VOLUME II MAY, 1931 No. 2

18. STARTERS

BY SØNCKE KNUDSEN.

Royal Veterinary and Agricultural College, Copenhagen.

(Translated by E. R. HISCOX, National Institute for Research in Dairying, Reading.)

(With 1 Plate and 1 Text-figure.)

CONTENTS.

	PAGE
Introduction	137
General characteristics of the lactic acid bacteria	138
Butter starters	142
History	143
Bacteriology	147
Preparation of starters	152
Treatment of starters in the dairy	154
Testing of starters	156
Influence of the milk on starters	157
Influence of "ripening" on the butter	159
Cheese starters	159
Starters for dietetic sour milk products	161
Bibliography	162

INTRODUCTION.

THE term "starters" as used in dairying is understood to refer to cultures of lactic acid bacteria which induce "souring" when inoculated into milk, cream or other product. Souring will usually occur without the use of starters, but the results will be erratic. With the help of starters, however, the reaction can be controlled so that it proceeds in a desired direction. The term generally suggests those starters which are used in the making of butter, but in its widest sense it must include *all* cultures which are used in the dairy for the production of any type of souring.

Starters are used chiefly for the making of:

(1) Butter;
(2) Cheese;
(3) Dietetic sour milk products.

All souring which is of any importance in dairying is what may be called "natural," *i.e.* such as could occur spontaneously under the right conditions. The use of starters does not therefore involve the introduction of *new* reactions, but merely ensures that the particular type of souring that takes place shall be the best of those which have been obtained spontaneously.

The various types of souring depend on the fact that the natural conditions for growth, especially the temperature, have differed, and as a result different bacteria have gained the upper hand and have determined the type of reaction. The use of the right starter, *i.e.* the right bacteria, for inoculation is therefore not in itself sufficient to ensure the correct souring, but care must also be taken that these bacteria are given the right conditions for development.

General characteristics of the lactic acid bacteria.

It is fitting that a preliminary review of the general characteristics of the true lactic acid bacteria should be given here, since these bacteria always constitute the starter.

Because of their great practical importance innumerable investigations of the lactie acid bacteria have been made from many and various points of view, with the result, unfortunately, that great confusion prevails in their classification and nomenclature. Not only are many different names given to the same organism, but the same name is used with different meanings by different investigators. In every individual case, therefore, it is necessary to indicate clearly what is meant by the name that is used. It should be especially emphasised that the term *Streptococcus lactis* is used sometimes to denote the whole of the group of streptococci which coagulate milk with the production of acid at ordinary temperatures, and sometimes to denote an individual species of the above-mentioned group as formulated by Orla-Jensen. In this monograph the term is used in the latter sense.

After a thorough study of the true lactic acid bacteria, Orla-Jensen(1) undertook a systematic classification in which not only their morphology but all their characteristics were considered.

Orla-Jensen's classification is used in this monograph, and a survey will, therefore, be given here of the position in this system of the bacteria which are of importance for starters, since this classification has brought order into a region where before there was chaos.

In suitable nutrient media the true lactic acid bacteria produce considerable quantities of lactic acid by the fermentation of various carbohydrates and higher alcohols. The various species make very special nitrogen demands, but none of them are satisfied with simple nitrogenous substances such as ammonium salts or single amino acids. They are Gram-positive, non-motile, and do not produce spores. They have no catalase, do not reduce nitrates and are indifferent to atmospheric oxygen, and in stab cultures, therefore, grow equally well throughout the whole stab without spreading over the surface.

The true lactic acid bacteria may be either rod-shaped or spherical. Whilst some of them produce lactic acid almost exclusively, there are others which produce, in addition, considerable quantities of other substances which Orla-Jensen denotes as by-products. On this basis they are divided into two groups, *A* and *B*, with the following genera:

Orla-Jensen's Scheme.

A. Produce only traces of by-products in addition to lactic acid.

Rod forms.

Genus: *Thermobacterium.* The optimum temperature generally lies at 40° C. or above, and the bacteria do not grow at temperatures lower than 22° C. They produce inactive or laevorotatory lactic acid. To this genus belongs, amongst others, *Thermobacterium helveticum*, which gives inactive lactic acid and is used as a starter in the making of Emmental cheese. To this genus also belong the bacilli which are found in yoghurt, and in all probability those which are used in the preparation of the so-called "acidophilus milk."

Genus: *Streptobacterium.* The optimum temperature is about 30° C. Inactive and dextrorotatory lactic acid are produced. To this genus belongs the species *Streptobacterium casei*, which is of importance in the ripening of many of the ordinary types of hard-pressed cheese.

Coccal forms.

Genus: *Streptococcus.* These always give dextrorotatory lactic acid. To this genus belong the species *Streptococcus cremoris* and *Streptococcus lactis*, which become dominant during the souring of milk at ordinary temperatures. Their optimum temperature is 25°–30° C., but they will still grow at 10° C. *Streptococcus cremoris* can form long chains in milk, but *Streptococcus lactis* is unable to do so. The deciding factor between the two species is, however, that *Streptococcus lactis* can ferment maltose and dextrin whilst *Streptococcus cremoris* cannot. *Streptococcus cremoris* is dominant in the starters which are used for inoculation into cream for butter-making and into milk for the preparation of the ordinary types of cheese.

B. Produce considerable quantities of gas and other by-products in addition to lactic acid.

Rod forms.

Genus: *Betabacterium.* These grow indifferently in milk but well in media which contain autolysed yeast. To this genus belongs the species *Betabacterium caucasicum* which is found in kefir.

Coccal forms.

Genus: *Betacoccus.* These produce laevorotatory lactic acid, a distinction from the streptococci which they resemble in appearance. They grow poorly as a rule in milk but well in media (including milk) to which autolysed yeast has been added. To this genus belong the bacteria which are found in ordinary starters and which are essential for the development of the aroma. The species which are termed *X*-bacteria, aroma bacteria, *Streptococcus citrovorus* and *Streptococcus paracitrovorus* are here called *Betacoccus cremoris*, according to the genus to which they belong.

In the arrangement of the many species of lactic acid bacteria Orla-Jensen has considered all their characters.

In the identification, the particular form of lactic acid produced and the power to ferment various "sugars" must be taken into account as well as the morphological characters.

In Table I is given the amount of lactic acid, calculated per mille, which the chief starter bacteria produce from the various "sugars" and from milk with and without the addition of autolysed yeast. The table also shows the type of lactic acid produced and the presence or absence of CO_2.

It must be added that the quantity of acid produced by a given species from a given "sugar" is not identical under all circumstances. The buffering action of the medium has a great influence, but if the relative quantities of acid produced by the fermentation of the various sugars are compared, the individual species are well differentiated.

It is evident that the identification of a definite species of the true lactic acid bacteria entails a considerable amount of labour. The authors of many publications have therefore been content to work with less well-defined groups, and in particular the name *Streptococcus lactis* is even now frequently used as a term for the whole genus which Orla-Jensen denotes as *Streptococcus*.

The genus *Betacoccus* (Orla-Jensen) comprises species which in appearance closely resemble the streptococci, *i.e.* are cocci which are capable of forming chains. According to the old nomenclature, which took into consideration the morphology only, these must also be denoted as streptococci. But if Orla-Jensen's classification is followed, it must be determined whether the lactic acid produced by these cocci is dextrorotatory or laevorotatory, as for streptococci and betacocci respectively. It is not possible, therefore, to determine, by the use of the microscope alone, whether or not a particular organism is a streptococcus.

Hammer was the first to give the names *Streptococcus citrovorus* and *Streptococcus paracitrovorus* to the betacocci which are found in starters. In spite of the fact that it is unfortunate to propose new names for bacteria the author is of the opinion that there is more justification for the name *Betacoccus cremoris*, so that these bacteria may be correctly placed in Orla-Jensen's scheme which, for lucidity, far surpasses all other nomenclatures hitherto proposed for the lactic acid bacteria.

The distribution of the lactic acid bacteria is so general that normally they will always be found in milk, which sooner or later becomes sour as a result of their activity. Mankind has, therefore, to a certain extent, become accustomed to regard souring as a normal characteristic of milk, and from earliest times sour milk has been used both directly as a food and for the preparation of dairy products.

This is chiefly because in many cases it has not been possible to keep the milk fresh, but its use has also been promoted by the facts that the taste

Table I. *Acidity calculated as lactic acid per mille.*

	Glycerine	Xylose	Arabinose	Rhamnose	Sorbitol	Mannitol	Laevulose	Glucose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Dextrin	Starch	Salicin	Milk	Milk with the addition of autolysed yeast	Optical activity of the lactic acid	CO_2 production
Streptococcus lactis (No. 4)	0	0	0	0	0	3·0	3·5	4·4	4·5	3·5	0	4·4	3·1	0	4·0	0	3·6	8·2	8·0	d	0
Streptococcus cremoris (No. 23)	0	0	0	0	0	0	4·0	4·1	4·5	3·5	0	0	2·7	0	0	0	2·9	7·1	7·5	d	0
Streptococcus cremoris (No. 85)	0	0	0	0	0	0	2·8	3·0	2·9	2·5	0	0	2·8	0	0	0	2·3	6·0	6·5	d	0
Betacoc. crem. (No. 1	0	0	0	0	0	0	0·5	1·6	0	1·6	0	0	1·9	0	0	0	0	0·7	7·5	l	+
Betacoc. crem. (No. 3)	0	0	0	0	0	0	0·4	2·1	0	2·0	1·5	0	2·2	0	0	0	0	0·8	5·6	l	+
Betacoc. crem. (No. 101)	0	0	0	0	0	0	0·6	2·6	0	2·4	1·3	3·8	3·0	0	0	0	0	4·1	10·0	l	+
Betacoc. crem. (No. 156)	0	0	0	0	0	0	2·5	3·5	1·5	2·2	3·7	4·4	2·8	0	0	0	0	6·1	8·8	l	+
Thermobact. helveticum	0	0	0	0	0	0	3·8	10·1	10·1	8·8	0	10·3	11·9	0	2·3	0	0	19·6	—	i	0
Thermobact. bulgaricum	0	0	0	0	0	0	2·7	3·2	1·8	0	0	0	7·9	0	0	0	0	16·4	—		0
Streptobact. casei	0·8	0	0	0	0	2·5	11·7	13·7	13·5	12·2	10·1	12·4	12·5	0	0·2	0	10·8	11·5	—	d	0
Betabact. caucasicum	0	0	14·2	0	0	0	3·8	7·0	0	7·7	0	0	0	0	0	0	0	0	—	i	—
Bact. from German acidophilus milk	—	—	—	—	—	—	—	11·7	11·9	10·9	12·5	10·2	11·9	—	2·9	—	0	13·9	15·6	l	—
Bact. from American acidophilus milk	—	—	—	—	—	—	—	11·3	12·2	9·2	10·9	10·7	9·3	—	3·7	—	4·8	11·0	12·9	l	—

produced by souring has been found to be quite agreeable, and that from a health point of view sour milk is equal to fresh.

Milk that is more or less sour has been used as a matter of course since olden times, and it is only within the last generation that technique has so far progressed that all the milk needed can be procured in a fresh state.

Sour milk is still produced for various purposes. The lactic acid fermentation, for example, is of the greatest importance in the making of cheese and butter, in which the quality of the finished product depends to a large extent on the souring which took place previously in the milk or cream.

According as the milk is stored at a higher or lower temperature, so different lactic acid bacteria develop, and the resultant souring varies. The most general type is that which occurs when the milk is allowed to stand at ordinary temperatures.

The starters which are used for the making of "Tykmaelk" (ordinary sour milk), for the ripening of cream and for adding to the milk in the making of most types of cheese, are those which sour at ordinary temperatures, and it is of these cultures that one generally thinks when mention is made of starters. But those used for sour milk products such as yoghurt and acidophilus milk, as well as for the manufacture of Emmental cheese, are cultivated at 37°–40° C.

Butter starters.

As has been mentioned, milk becomes sour naturally; but even if the same temperatures are used it by no means follows that the result will be the same in all cases. Differences are particularly apparent in the smell and taste, chiefly because of the development of different micro-organisms. Thus bacteria which produce substances imparting various, often disagreeable, tastes and smells can develop in addition to the lactic acid bacteria. Even if all bacteria other than the true lactic acid bacteria can be successfully suppressed there are so many different strains that the taste and smell of the sour milk may vary considerably. This is not entirely due to the fact that the different strains produce different quantities of lactic acid, since the same result *cannot* as a rule be obtained by adding corresponding quantities of chemically pure lactic acid to the milk. Differences in flavour depend primarily on small quantities of other substances—by-products—which these lactic acid bacteria produce conjointly with their chief product, lactic acid. The quantity and the nature of these by-products evidently vary for the different bacteria, so that at times an approved flavour is obtained by spontaneous souring, whilst at others the taste is disagreeable. The chemical composition of these flavour-producing substances is not known in the majority of cases.

Properties other than the flavour imparted to the sour milk vary with the different lactic acid bacteria, as, for example, their casein-splitting power; and whilst this power is of decided importance for the bacteria concerned in the ripening of cheese this property is unnecessary in the making of butter.

From the foregoing it will easily be seen that it may be a great advantage to use a starter instead of being satisfied with fortuitous souring, which though it may often be satisfactory, yet just as frequently may give exceedingly bad results.

When bacteriology had reached a certain stage in its development it was quite naturally reasoned that, since different lactic acid bacteria gave different results, the *best* strains should be isolated and used exclusively. This reasoning was supported by the excellence of the results that this method had given in the breweries where a certainty hitherto unknown in the work was being acquired.

It has gradually become apparent that the souring of milk, like most of the fermentations used in practice, is more complicated than had been at first assumed. The best results are obtained, not by the use of a single organism in pure culture, but by the interaction of various organisms, so that the use of a pure culture is not satisfactory.

Although the scientific preparation of starters has encountered many difficulties, all of which are not overcome even now, yet their use has been of very great technical, as well as economic, importance. The starter which has by far the greatest importance is that used for the ripening of cream in the making of butter, and this has therefore been most fully investigated, and will be treated here in greatest detail.

History.

The use of starters had been adopted long before anything was known about bacteriology. The cause of their activity was unknown, but experience had shown that, by the inoculation of fresh milk with small quantities of sour milk, properties could be transmitted to some extent from one portion of milk to another.

Since the rise of bacteriology in the middle of the last century the bacteria which develop in milk have gradually been found. At the end of the 'eighties scientists began to investigate the question of souring from various points of view, with the result that in 1890 the main course of the reaction was demonstrated almost simultaneously, but quite independently, by Conn (2) in U.S.A., Storch (3) in Denmark and Weigmann (4) in Germany. These workers laid the foundations for the preparation and use of starters, a practice which, in the course of a few years, spread throughout Denmark, where it is now in constant use, as well as in a number of other butter-producing countries.

In 1895 the use of these starters was general in Denmark, and the benefits gained by the practice are summed up in a report from the Danish Research Laboratory (No. 32, 1895), from which the following is quoted since it gives a good picture of the history of the use of starters in Denmark.

> The very significant growth of our dairying industry during the last decade, from a comparatively modest branch of agriculture proper into the most important industry in the land, naturally demands more and more insistently that attention shall be directed towards further progress if the position acquired by efficiency and hard work is to be maintained.

The aspect of dairying which has attracted the greatest attention is butter production, and it is in this branch that improvement in quality and increase in quantity have been greatest. The increase in quantity, due in part to the increase in the number of cattle and the rising average milk yield per cow, and in part to the more judicious use of the milk as a result of the introduction of the centrifugal separator, can be comparatively easily proved, but it is far more difficult to point out the progress in quality. Yet there is no doubt that in this direction also there has been a considerable general advance, and amongst the factors which have contributed most to this progress, mention should be made not only of the general improvement in the methods of manufacture, but, in particular, of the more intelligent treatment of the cream during the period of ripening, a factor of the greatest importance where the production of butter of the finest quality for export is concerned.

Only scanty, and at times somewhat vague, information is available concerning the early methods used for the ripening of cream. The most general method was the so-called "Spontaneous souring" in which the milk, on standing, simply became sour naturally without the addition of any special souring medium. Although spontaneous souring did not appear to present any particular difficulty, it nevertheless demanded great care, especially with respect to the temperature conditions, and only the efficient and careful dairymaid could be tolerably certain of getting the cream ready for churning at the right time. Different methods of assisting the spontaneous souring were tried in order to counteract the difficulties resulting from the time of year and the state of the weather. The tubs of cream were placed in a sheltered place which in winter was heated; at times part of the cream was heated and poured into the remainder; but more often the cream was warmed in the tub either by the addition of hot water with vigorous stirring, or by plunging an earthenware or tin jar filled with hot water into it. One of the best ways of warming the cream was to add warm new milk, 12–24 hours before the churning, a method which was used more particularly in the winter when there was only a small quantity of milk. These different practices, which were all designed to create a suitable temperature, served, in conjunction with constant care in the stirring of the cream, to hasten the souring. If premature souring seemed likely to occur different methods were adopted to delay the further development of acidity. Usually the cream was distributed into several tubs, and freshly skimmed cream added; sometimes a portion of the cream was withdrawn, cooled down and again added, or the whole bulk was poured into another clean, well-cooled tub. But however much care was taken, and whatever measures were adopted, spontaneous souring was often a failure, mainly because the use of the thermometer was unknown in the dairy. This was first introduced in 1860 by Segelcke;...afterwards its use became more and more general and since that time the thermometer has been invaluable in the control of dairy processes.

The addition of special souring media was another method used....Feilberg (1861), after his travels in Holstein, reported that if for any reason the souring of the cream did not proceed quickly enough, the formation of acid could be hastened by the addition of a few cans of buttermilk. Segelcke...also stated that, although buttermilk was used for the ripening of cream, many experienced dairymaids did not approve of the practice....

It is not clear why buttermilk which had previously been considered a temporary substitute for a sourer by recognised dairy folk, should later, in fact until a few years ago, be regarded as a good souring medium, but it is reasonable to assume that formerly the buttermilk was so heavily infected that it could not be a good souring medium, since the cream, whilst standing, had been exposed to the air and was often already slightly sour when skimmed. Later, after the introduction of water, ice and the centrifugal separator, the buttermilk was much cleaner and therefore made a better souring medium. In fact, it was only after the introduction of this system of dairying that buttermilk was used to any great extent as an aid to the ripening of cream.

As a rule fresh buttermilk was used, but occasionally, especially when the cream had not soured correctly and uniformly, buttermilk from the previous day was taken, since it was

the practice to set aside enough for use if the new buttermilk should not be sufficiently good, a precaution which, as will be discussed later, is also taken with other souring media.

From the close of the 'fifties until well into the 'seventies the use of sour cream seems to have been the method most generally adopted by the large and best-managed dairy farms, whilst spontaneous souring was used by the smaller dairies where there were, as a rule, only one or two churnings in a week. By about 1860 most of our best-known dairies were using sour cream....The sour cream, as generally used for ripening, was not specially prepared; a part of the cream which was ready for churning was simply added to the sweet cream and then heated to a suitable temperature. It was only after the introduction of the thermometer and of improved dairy records by Segelcke and Friis that definite quantities of starter were used for definite quantities of cream. Formerly the amounts had been taken at random, a procedure to be deprecated, since the ripening, as well as the churning, was extremely irregular.

The use of sour milk prepared from sweet milk and a certain proportion of warm water which were mixed and set aside in a sheltered place for about a day until the mass had become thick and smooth—*i.e.* the so-called "new sourer"—was general in Holstein in the 'sixties. The method is mentioned by both Segelcke and Feilberg, after their journey in Holstein. Similarly Segelcke mentioned...in 1878 that sour milk could be used as an inoculum for cream, in 1879 he advised the use of sour milk as a starter in order to hinder the transference of infection from one cream vessel to another, and finally, in 1881, stated "there is a probability that the use of buttermilk may introduce into the cream things which are not beneficial for the ripening." In the beginning of 1881 Segelcke used the expression "new sourer" for the first time. Winkel, at the end of 1881, mentioned a starter produced by him at Aunsbjaerg, which in all essentials resembled that mentioned by Segelcke and Feilberg in 1861–3. In both cases a mixture of milk and water was used, but whilst the latter added warm water in order to heat the milk, Winkel heated the mixture of milk and water to a definite temperature.

In the Danish dairies the so-called "new sourer" was usually prepared from fresh, clean-smelling, sweet milk which was heated to a certain temperature and then set aside to sour spontaneously, either in a sheltered place or in an insulated vessel until, after 12–20 hours' time, it had become sour and quite uniform. In more recent years freshly skimmed milk has been used for the preparation of the "new sourer," but in all other respects the treatment has been similar to that for the whole milk.

The ripening obtained with buttermilk and with sour cream did not satisfactorily fulfil the requirements, as is evident not only from verbal statements and writings in the agricultural press, but also from the fact that a full discussion of the question of souring was suggested as the subject of a thesis. This is a reference to the prize of 1000 kr. offered by the Scandinavian Preserved Butter Company in the year 1878, "for a thorough investigation of the conditions which influence the souring of cream, for it must be recognised that the way in which this is usually carried out in the dairies makes it extremely difficult to deliver a uniform product." In the theses submitted various suggestions were made, but though these were studied by many people no satisfactory solution was found within the given time.

Whilst up to this time the ripening of the cream had been almost exclusively based on the experience gained in practical dairying, results of bacteriological research began to be applied in practice in the 'eighties. In 1884 Storch undertook some bacteriological investigations of certain faults in butter, which led him, in 1887, to embark on an investigation of lactic acid fermentation itself, with special consideration of its importance in practical dairying. Not only did he isolate and grow in pure culture a series of lactic acid bacteria, but he also endeavoured to find out the influence of individual bacteria on the souring by inoculating them into sterile milk or cream, just as he tried by means of churning experi-

ments on a small scale to arrive at a knowledge of the importance of various strains of lactic acid bacteria for the taste and aroma of butter.

By the time that the first pasteurising experiments were carried out at Duelund in the year 1888 Storch had progressed so far with his investigations that his pure cultures of lactic acid bacteria could be used as a souring medium for the pasteurised cream. The pure cultures were sent in tubes from the laboratory to the dairy farm where they were propagated by C. O. Jensen for use with the cream. The first experiment was a great success; it is true that Storch's pure culture did not give such fine-flavoured butter as that produced after ripening with buttermilk from a dairy which at the time was supplying particularly good butter, but it was free from souring faults. The results of Storch's researches, which were continued until 1890, are found in the 18*th Report of the Danish Research Laboratory*.

In the course of about a year after this no less than four Danish manufacturers offered to supply acid-producing cultures to dairymen....At first these were all sent out in the liquid state, in which form they are most easily transplanted. Since it soon became apparent that long transport under unfavourable conditions of temperature often had a deleterious effect upon the cultures, the manufacturers endeavoured to produce them in the dry state....Commercial starters have gradually been more and more appreciated in the dairies, and the time is not far distant when they will practically supersede the older means of souring....

It is undoubtedly a step forward that good starters can be produced on wholesale lines; the effect of this is not only that dairies need no longer be in doubt as to how they shall secure a good ripening of the cream, but they also have a guarantee that the starters received at different times are uniformly good. With the scientifically controlled production of starters which now exists there is obviously a much greater possibility of improvement than was formerly the case, and dairying has good reason to thank the men who devoted their time and energy to the solution of this worthy problem.

A development similar to that just described for Denmark took place at the same time in other dairying countries, so that it became a general practice to pasteurise the cream and then ripen it with the help of a starter, thereby attaining a sureness in the work and a uniformity in the product such as had not been known hitherto.

The chief findings of the classic works on cream ripening in 1890 show that different strains of the ordinary lactic acid streptococci induce different types of souring in milk. Some give a clean sour taste, others give in addition an unpleasant tang, and still others give a mildly acid but strongly aromatic taste.

It was the last type which was worth obtaining in pure culture and using as starter, and as long as such cultures could be kept pure and active, good results should have been assured. When less good results were obtained it was believed that they might be attributed to impurities or to a weakening of the strain.

The use of starters on this basis quickly gained a very large practical and economic importance, and experience of them in the dairy strengthened the theory.

The view was generally held, however, that spontaneous souring, when it proceeded favourably, would produce a stronger aroma than could be obtained with starters which contained only a single bacterial strain in pure culture,

and efforts were made to find other organisms which would increase the aroma when grown with the bacteria originally found in starters.

In the years 1918 and 1919 the first accounts were published of investigations which showed that two different types of bacteria are found in good starters, *i.e.* the old familiar type, *Sc. cremoris*, and bacteria which later were referred to the family *Betacoccus* (Orla-Jensen).

As in 1890, so also in 1919 this discovery was made in three different places, by Boekhout and de Vries (5) in Holland, by Hammer and Bailey (6) in U.S.A. and by Storch (7) in Denmark. In reality Storch had never discontinued his study of the bacteriology of starters since he had begun it in the 'eighties, and there is no doubt that it had long been clear to him that the classic theory was not wholly satisfactory. He had found the above-mentioned betacocci quite early in his studies, but he had never been able to persuade himself to publish these particular researches because he found that something was still lacking which was necessary for the full understanding of the question; thus the above-mentioned work was only published after his death, amongst his posthumous writings.

Whilst the fuller understanding of the ripening of cream, afforded by the various discoveries of 1919, is just as important scientifically as the investigations of 1890, its economic and practical significance cannot in any way compare with that of the classic investigations. The classic theory of souring, in spite of its deficiencies, was sufficiently accurate to serve as a basis for work. But it has not been slavishly followed in practice, and its benefits have thus been enjoyed whilst its shortcomings have been ignored. This has parallels in many other fields and is due to the fact that every theory is merely an approximation.

Bacteriology.

Most of the starter bacteria are different varieties of *Streptococcus cremoris* (Orla-Jensen) similar to those which Storch, Weigmann and Conn found in 1890.

The streptococci which develop during the first stages of the spontaneous souring of milk, *i.e. Streptococcus lactis* (Orla-Jensen) are, on the contrary, scarcely normal inhabitants of starters. When these are prepared by the spontaneous souring of milk *Streptococcus cremoris* gradually supersedes *Streptococcus lactis*.

As far as can be ascertained, *Streptococcus cremoris* and *Streptococcus lactis* induce the same type of souring in milk, and it might therefore be thought that strains of both these species might be suitable. Since, however, *Streptococcus cremoris* is the organism characteristic of good starters, the presence of *Streptococcus lactis* is not as a rule anticipated, and when it is occasionally met with it is regarded as a harmless impurity. It should be mentioned, however, that in Henneberg's (8) experience starters do not so easily become slimy when they contain both *Streptococcus lactis* and *Streptococcus cremoris*, as when only the latter is present.

Streptococcus cremoris grows well in ordinary milk. Its optimum temperature lies between 25° and 30° C., but it grows well even at 10° C. The rate of growth varies with the different strains even when the cultures are fresh and vigorous. Most strains, however, produce a little more than 0·5 per cent. lactic acid and coagulate the milk in less than 24 hours. The reaction of the milk then corresponds to a *p*H of about 4·5, but *Streptococcus cremoris* apparently ceases to grow even when the *p*H has reached a value of about 5. The increase in acidity which afterwards takes place, and continues, though very slowly, for several days, is due to bacteria which had already begun to grow when the milk was less acid.

It should, however, be mentioned here that, as W. van Dam (9) and O. Svanberg (10) have shown, it is not the hydrogen-ion concentration itself which affects the growth of the bacteria under these conditions but lactic and acetic acids as such.

For the fermentation reactions see Table I.

A number of strains of *Streptococcus cremoris* can be isolated which differ from one another in size and in the length of the chains.

They differ also in rate of growth and consequently in the rate of souring. Further, whilst some possess a very strong casein-splitting power, in others it is almost entirely absent. The reaction towards bactericidal substances also varies; some strains are strongly inhibited, others scarcely at all (11,12).

Finally there are some, though few, strains which grow poorly in very clean milk, and it is difficult for them to start unless small quantities of other substances are added, substances which are produced in milk and other media by the growth of various organisms (12).

It was believed for about 30 years that pure cultures of the bacteria, which have just been described, should give the ideal ripening of cream. It may be well, therefore, to give an account of the differences between a pure culture of these bacteria (*Sc. cremoris*) and a starter which has the characteristics desired in practice.

In such a comparison the marked difference in smell and taste will be noted first. A pure culture of *Streptococcus cremoris* never acquires the full and aromatic, mildly acid flavour of a good starter, but always tastes sour and insipid in comparison.

Although the pure culture creates a more acid taste than that produced by the starter, it is found on titration that the acidity of the latter is equal to or greater than that of the former. If the titration is the same for both, then the hydrogen-ion concentration is less (*p*H is greater) for the starter than for the pure culture, which explains why they do not taste equally sour. When, as in this case, during the souring of the same milk the same titration but different *p*H is obtained, it may be concluded that it is not the same acid which is formed in both instances. In the pure cultures the quantities of acid other than lactic acid are scarcely appreciable, and it may therefore be concluded that in starters one or more weaker acids must be present in addition

to the lactic acid. Chemical investigations show that good starters contain not only lactic acid, but also the weaker acetic and carbonic acids, and the latter in particular is important in producing a mildly acid taste in spite of the high titration. Propionic acid is also present in very small quantity (13).

Another difference between pure cultures of *Streptococcus cremoris* and starters is that whilst the lactic acid in the former is entirely dextrorotatory, in the starter some laevorotatory acid is found although the bulk is dextrorotatory. This shows that good starters cannot be pure cultures of *Streptococcus cremoris*, although these bacteria certainly are present in the largest numbers, but they must contain in addition at least one other kind of bacteria.

As was mentioned in the discussion of the history of the development of starters such organisms were found simultaneously in three different places. Boekhout and de Vries called them aroma bacteria. Storch, too, was content with a provisional name for them, namely *X*-bacteria; Hammer called different types *Streptococcus citrovorus* and *Streptococcus paracitrovorus*. In this paper they are termed *Betacoccus cremoris*.

Now that they are known, these bacteria can be isolated from every good starter, and by placing them with *Streptococcus cremoris* a mixed culture can be obtained whose properties correspond exactly with those of a normal starter. There can therefore be no doubt that it is these two types which together constitute the bacterial flora of starters.

These aroma bacteria are chain-forming cocci similar to *Streptococcus cremoris*, but usually somewhat more slender. They do not obviously alter the milk in which they are grown, and it is only after a long time that titration demonstrates the production of very small quantities of acid, which is for the most part acetic acid (volatile acid).

No particular aroma is noticeable in such milk, and the growth of the bacteria is on the whole poor, which undoubtedly is the reason why they were not found long ago. It was simply assumed that there was no growth when the milk was not obviously changed and the cultures were therefore discarded without further examination.

As long as only those characteristics of the aroma bacteria determined by the original investigations in 1919 were known, it was difficult to understand how they could play an essential part in the development of the aroma of starters, since in pure culture they had no appreciable action on milk. The key to this problem was found when it was shown by the investigations of Ayers and Mudge (14), together with those of Orla-Jensen and his collaborators (15), that the aroma bacteria were able to grow vigorously and produce large quantities of acid if only they were placed in the right medium.

Milk is really not at all a suitable medium for these bacteria, for they grow only very poorly in it and do not exhibit any of their characteristic properties. The deficiency in milk can, however, be rectified by the addition of various substances such as autolysed yeast, which are effective even in small quantities. The aroma bacteria are then able to grow well and their properties can be

examined under favourable conditions and those which are characteristic for the bacteria determined. Orla-Jensen (15) showed that they belonged to the betacocci, and Søncke Knudsen and Sørensen (12), after having investigated a large number of these bacteria isolated from starters, called them *Betacoccus cremoris* according to the position they naturally fell into in Orla-Jensen's classification.

It is thus demonstrated that *Betacoccus cremoris* may display great activity under the right conditions, and can produce, in addition to lactic acid, a number of other compounds amongst which the substances responsible for the characteristic aroma of starters and butter may be found.

Since starters are cultivated in ordinary milk in which the betacocci do not display any noteworthy activity, their great effect can scarcely be explained except by assuming a symbiosis between the betacocci and the streptococci, in which the latter produce degradation products without which the former cannot develop vigorously. The best-known example of such a symbiosis is the kefir fermentation, in which the rod-shaped lactic acid bacteria and a yeast grow together in milk, with the result that acid is produced in a quantity corresponding to a titration of about 200 c.c. of tenth normal caustic soda solution per 100 c.c. of the product, although neither of the two organisms alone gives appreciable quantities. The yeasts yield certain substances which enable the lactic acid bacteria to develop and produce the large quantities of acid. Lactic acid bacteria from kefir can be made to give equally large quantities of acid by adding to the milk, in the form of sterile autolysed yeast, those substances which are formed during the symbiosis.

The same thing occurs with the betacocci in starter though it is not so obvious; they grow only feebly when they are alone in milk but thrive in starter because the streptococci produce the substances they need, which are lacking in the pure milk.

The various bacteria have a very great influence on the growth of one another, and therefore it is clear that their development cannot be the same when grown in pure culture, as when they are cultivated in a dairy where infection is liable to occur.

It is of special interest that, as was pointed out by Storch (7), the various strains of *Streptococcus cremoris* and *Betacoccus cremoris* do not all thrive together equally well.

The influence of the streptococci on the growth of betacocci is greater than the converse, since the streptococci constitute about 90 per cent. of the total bacteria in starters, whilst the betacocci form only 10 per cent.

The action of the streptococci is very complex. They favour the betacocci by producing substances which promote their growth and enable them to play a part in the starter, but at a later stage the acid, produced for the most part by the streptococci, limits the growth of both streptococci and betacocci.

When the *p*H, which in normal milk is about 6·7, is reduced to about 5 by the production of acid, the growth of the betacocci as well as of the streptococci

is arrested, and since the streptococci produce the bulk of the acid, they virtually set the limits for the multiplication of the betacocci. Slow development of acidity therefore encourages the growth of the betacocci, which is in good agreement with the practical experience that betacocci thrive best in company with slow-growing streptococci. But the more slowly the souring is allowed to proceed, the greater is the danger of infection, so that in the process of souring many factors must be taken into consideration.

As is evident from the foregoing, there can be no doubt that the betacocci are responsible for an essential part of the aroma of starter, and, according to all practical experience, of butter also. The nature of the compounds on which this aroma depends is of interest. The substances which the betacocci produce in large quantities in addition to lactic acid are acetic and carbonic acids. It is scarcely credible that acetic acid should have any marked influence on aroma, neither can it be imagined that CO_2 gives the aroma. On the other hand there is no doubt that CO_2 is the essential cause of the mildly acid flavour, which is always emphasised as characteristic of good starters. This is connected with the fact that the CO_2 in the solution raises the titration value without a corresponding rise in the hydrogen-ion concentration (*i.e.* without a fall in the *p*H). For example (12) 100 g. of a starter required 102 c.c. of tenth normal caustic soda solution for titration, whilst the *p*H was 4·48. After the CO_2 had been removed by aeration the titration fell to 87 c.c., whilst the *p*H was found to be almost unchanged at 4·46.

It is known that in various beverages CO_2 gives a pleasant refreshing taste so that it might quite reasonably improve the flavour of starters. Further evidence of this is found in the fact that a starter from which CO_2 has been removed by stirring or aeration has an inferior flavour. At the same time CO_2 cannot be assumed to be an important factor in the aroma of butter, and doubtless the betacocci must produce other more aromatic substances. The demonstration of such substances will always be difficult since only small quantities are concerned, but from the researches of van Niel, Kluyver and Derx (16) there is reason to believe that the aroma of butter, or at least an essential part of it, is due to *diacetyl*, a compound which Schmalfuss and Bartmeyer (17) found in starters.

The formation of "by-products" in starters is best understood by assuming that two associated fermentations take place:

(1) The true lactic acid fermentation in which the sugar is converted exclusively into lactic acid.

(2) A fermentation which yields CO_2, acetic acid and small quantities of other products, amongst which the special aromatic substances may be found.

The former, the true lactic acid fermentation, can be brought about by all lactic acid bacteria when they are able to develop normally, and it is the only fermentation possible for many of them. This applies to all members of the main group of true lactic acid bacteria which do not give by-products, *e.g.* the streptococci.

The betacocci can induce the second fermentation conjointly with the first and in so doing consume some of the acid. It appears that the lactic acid bacteria obtain their initial energy by converting the sugar into lactic acid, the presence of which liberates citric acid in the milk. The increase in acidity inhibits further growth except in those cases in which the bacteria are able to utilise the lactic and citric acids for the second fermentation.

It has always been found that the quantity of the by-products formed by a definite strain of lactic acid bacteria varies greatly, and often the power of bringing about these changes is entirely lost; but both facts are in agreement with the hypothesis that two parallel fermentations are in progress.

Hammer came to the conclusion that it is the citric acid, and perhaps also the lactic acid which the aroma bacteria utilise. The author (12) has been able to confirm Hammer's results in so far that the by-products of the second fermentation are formed from these compounds, and citric acid seems particularly easy to split in this manner. On the other hand the aroma bacteria do not appear to feed on citric acid primarily. If they are inoculated into three portions of a medium favourable for bacterial growth the first of which contains sugar, the second citric acid, and the third both sugar and citric acid, it is found that they grow poorly with citric acid alone, but give by far the greatest evolution of gas when both sugar and citric acid are present. This confirms the view that the growth and activity of these bacteria depend primarily on the presence of sugar. It is only when large numbers have developed, and they are unable to tolerate more acid, that they resort to the second fermentation in which the citric acid in particular is attacked. The second fermentation proceeds best with an acid reaction (low *p*H), and therefore starts late.

Preparation of starters.

It is evident from the foregoing that starters can be obtained in many ways, but fundamentally they can all be traced back to spontaneous souring of milk.

If it is desired to procure a starter by means of spontaneous souring a portion of the soured milk must be transferred to fresh milk daily for a long time before it is safe to assume that the product is tolerably constant. At first the inoculation should be made into raw milk as for spontaneous souring, so that the desirable organisms in this milk may have an opportunity of flourishing; but when, after a few days, a sour milk of the right taste and smell has been obtained, the inoculation can be carried on in pasteurised milk to avoid the introduction of new organisms. Those already present will live together and eventually by their rapid growth the desirable bacteria will suppress any foreign organisms that may still remain. In many cases a satisfactory starter can be made in this way, but it may happen that it does not remain good, and then another sample of milk must be set aside for spontaneous souring and similarly treated. If, on the whole, good results are obtained by this method it is because the starter organisms adapt themselves naturally to

milk, and by frequent re-inoculation therefore gain the mastery. It is easy to understand that they exclude from the milk many bacteria which cannot tolerate acid, but they also inhibit a number of acid-tolerant organisms (rod-shaped lactic acid bacteria, yeasts and moulds) which grow more slowly than the starter bacteria proper; this is why frequent transference is necessary. The temperature should be in the neighbourhood of 20° C., although good results can be obtained at higher and lower temperatures.

This is the original method and it is still able to give good results, but before such a starter is used it is absolutely necessary to cultivate it in pasteurised milk for some weeks and prove its good quality throughout that time.

At the same time it is not possible to be quite sure of such a starter, and it may therefore be plated out on a medium which favours the growth of starter bacteria. From the colonies which develop pure cultures may be made in milk, and their freedom from impurities tested by investigation of these milk cultures. By combining several of these pure cultures of streptococci and betacocci a good starter of assured purity can be recovered.

But even when pure starters are used their satisfactory quality must be verified by means of the taste and smell, for it is not possible to forecast these properties by means of bacteriological investigations alone. Individual bacteria may often be so weakened by continued propagation in pure culture that they are not able to hold their own in competition with other bacteria in the starter.

The milk is another factor which makes for uncertainty, since, as will be discussed later, it may show small differences which react on some lactic acid bacteria but not on others.

The preparation of starters is now left to experts who have specialised in this work. In the dairy therefore it is not a question of making a new starter, but rather of carrying on a culture which has been bought or procured in some other way. The methods of sending starters from special factories (or laboratories) vary. If the transit need not take more than a few days the milk culture itself is sent in a flask. This may be prepared either by pasteurising the milk in the flasks themselves and, after cooling, inoculating each individual flask with starter, or by inoculating a large quantity of pasteurised milk and later, at a certain stage in the ripening of the starter, distributing this in well-cleaned flasks. When the milk coagulates there is no further growth of the lactic acid bacteria, and the longer the milk is allowed to stand in this condition the greater will be the number of bacteria that will die, so that the culture becomes less vigorous. This is a fact that ought to be considered and the cultures should be dispatched before they are fully developed so that the last stage of the growth takes place during the transit. The first inoculation of a starter which has been sour for some days should be heavy because its vigour has diminished.

When the transit takes a longer time, the cultures will be weakened still more, and in this case it is preferable to dry them on an inert substance, *e.g.*

milk sugar, milk powder, starch, etc. These materials must be sterilised before the cultures are distributed upon them, and the drying must be carried out in a closed vessel at a low temperature, *e.g.* in a vacuum desiccator.

Such a dried culture must be revived before it is used, and this is done by transferring some of the powder to pasteurised milk which is allowed to stand at about 20° C. As a rule this souring will proceed quite slowly, and when the milk is sour it is inoculated into a new portion of pasteurised milk. This propagation is continued until a culture is obtained which is so vigorous that it will sour milk as quickly as required. The dry cultures are very much weakened, but this no doubt occurred to some extent during the preparation. They undergo no further change for a long time, however, and can be used in the above way even when they are several years old.

At the same time it is troublesome to revive dry cultures, so where possible the liquid milk culture should be given preference.

Treatment of starters in the dairy.

In the dairies a certain portion of *freshly ripened* starter is added to the cream each day, and the starter must therefore be cultivated in the dairy itself.

In many dairies an endeavour is made to preserve the starter as long as possible and what has been called "mother starter" is often used, *i.e.* the starter is inoculated into a small quantity of milk, *e.g.* 0·5–1 litre, since the various precautions can be more easily taken with small quantities than with large. The mother starter for the following day is inoculated with a small quantity of the ripened mother starter and the remainder is used for the inoculation of the large portion of "bulk starter," which should be ripe enough to add to the cream on the following day.

The use of mother starter, though it involves more work, certainly diminishes the risk of infection of a starter. In many places mother starter is no longer used and only bulk starter is cultivated in the dairy; from this the starter for the next day is first inoculated, and the main bulk is afterwards used for adding to the cream. The actual cultivation of mother starter and bulk starter is the same in principle, it is only the quantity which varies. The improved apparatus now available for the regulation of the temperature of larger quantities of starter is in some measure responsible for the fact that now there is seldom occasion to use mother starter, but the chief reason is that it has become more and more general to obtain a new culture fairly frequently from a laboratory or other agency.

As mentioned above, there are some dairies which keep the same starter as long as possible, and there are sundry instances of dairies in which the same starter has been cultivated with good results for 20 to 30 years. In contrast to this there are many dairies where a new starter is obtained regularly once a week, so that the starter shall not be in the dairy long enough to become

infected. Between these two extremes, the regular weekly change and the constant use of the same starter, all intermediate stages are found.

Whilst it will be readily agreed that a dairy must procure a new starter as soon as any fault can be found with the old, it is much more difficult to decide whether it is right to replace the starter while it is still proving satisfactory. Even though it be true that the frequent use of a new starter provides a certain security against spoilage due to infection, yet a starter is such a complicated system of organisms that it must be best to make as few changes as possible.

Naturally, in a dairy care must be taken that a new starter is obtained as soon as the old becomes faulty, and everything with which the new starter comes into contact must be thoroughly heated so that nothing from the old starter may be able to develop in the new. The mixing of old and new starters is not a wise procedure, and even though no harm may result if the old starter is not faulty, such a method cannot serve any good purpose.

When a starter from a laboratory is inoculated for the first time in the dairy it is generally found that the souring proceeds slowly and not altogether as one would wish. Presumably this results from the weakening of the bacteria during their prolonged stay in an acid liquid. It may also be due to some extent to the fact that the cultivation of the starter in one place is not exactly the same as that in another, so that identical strains of bacteria do not predominate in all cases. In this connection it must also be remembered that even small differences in the milk may have great influence on the starter and that after a few inoculations in a different milk a starter may be completely changed.

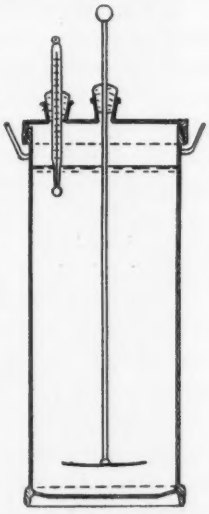


Fig. 1. Container for use in the preparation of starter. (After Rosengren.)

The cultivation of starter in the dairy aims at retaining the vigour of the bacteria and avoiding contamination with foreign organisms. To fulfil the latter condition, the milk is pasteurised, and after cooling so kept that no re-infection is possible. The milk should be pasteurised in the vessel in which the souring is to take place, and care must be taken that after the pasteurisation nothing which can infect it comes into contact with the milk; the stirrer and thermometer in particular must be well scalded before they are placed in the milk. The best arrangement, according to Rosengren, is the container shown in Fig. 1. The cover is removed at the time of inoculation, but otherwise the vessel is closed even during the stirring and the stirrer cannot be picked up without removing the lid, so that there is no temptation to use it for any other purpose after it has once been pasteurised with the milk.

If the bacteria are to remain permanently active they should not be left too long in the sour milk. The number which are viable reaches a maximum at the time of coagulation, and from that moment the bacteria begin to weaken.

It has been proposed that starter should be used just before it coagulates in order that the cream shall be inoculated with actively growing bacteria which can react immediately, but at the present time it is general to use coagulated starter in which bacterial growth is suspended and is only renewed after some time.

It must be clearly understood that the longer a starter is allowed to stand after coagulation has taken place the weaker will the culture become. On the other hand, even after the culture has stood for some weeks, so many bacteria may still be alive that a serviceable starter can be recovered from it by repeated inoculation. Bacteria do not all die at the same rate and the betacocci are found long after the streptococci have died out; it was because of this that Storch succeeded in isolating these bacteria.

For the starter bacteria, both streptococci and betacocci, the optimum temperature lies between 25° and 30° C., but it is not customary to hold the starter at such a high temperature since the souring can be more easily regulated at a lower temperature at which growth is slower. A temperature of about 20° C. is most frequently used, but higher and lower temperatures are sometimes preferred. Although it is not possible to cite any definite temperature as the correct one, none the less it must be controlled and maintained at the same level from day to day, for it must be emphasised that starter is a mixture of organisms in a state of equilibrium which is upset as soon as the method of cultivation is changed.

In order that the temperature used for the cultivation of the starter may be kept sufficiently constant, it is necessary to use a thermostat which is big enough for the comparatively large quantities that are usually prepared in the dairy.

According as a higher or lower temperature is selected for the cultivation of the starter a correspondingly less or greater quantity must be used for the inoculation, in order that the starter shall be "ripe" at the same time on the following day; 0·5–1 per cent. of inoculum will usually be suitable.

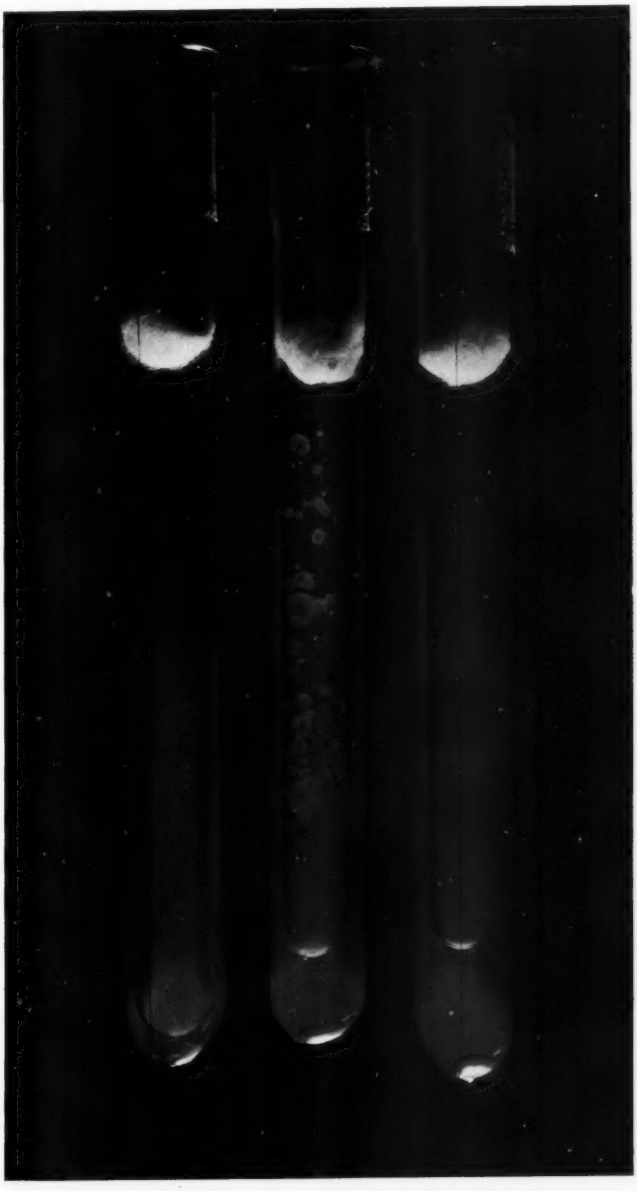
Care must be taken that the starter does not stand for a long time in a sour (coagulated) condition so that the bacteria become weakened. On the other hand, it will be difficult to control the quality of the starter if it is not allowed to ripen until the taste and smell and titration can be used to indicate its condition.

Testing of starters.

The organisms with which there is danger that the starter may be contaminated must naturally be acid-tolerant. There is therefore no possibility of the development of the putrefactive bacteria which so commonly occur.

The chief organisms found in starters are:

(1) True lactic acid bacteria which are not normal inhabitants of starter.
(2) Tetracocci.
(3) Yeasts.
(4) Moulds.



One infected and two pure starters tested by means of Burri's smear culture method.

Of the undesired true lactic acid bacteria the rod forms can be demonstrated by the titration of an 8-day culture. Since lactic acid-producing cocci do not give in milk more acid than corresponds with a titration of 120 c.c. tenth normal caustic soda solution per 100 c.c. of milk, using phenolphthalein as indicator, a titration which is definitely higher shows that rod-shaped lactic acid bacteria are present.

The demonstration of harmful lactic acid-producing cocci is far more difficult, but it is generally safe to assume that if the taste and smell of the starter are satisfactory, lactic acid-producing cocci which can give an objectionable taste or smell to the butter will rarely be found.

A caramel and malty flavour may be traced to certain strains of *Streptococcus lactis*. Sliminess may also be due to streptococci and may almost be considered a sign of their degeneration.

Contamination with organisms other than true lactic acid bacteria can be demonstrated by plating out on suitable media on which the latter cannot grow. For this purpose the following media may be used:

(1) Peptone agar without the addition of sugar.
(2) Asparagin-glucose agar.

True lactic acid bacteria fail to grow in the first because of the lack of sugar, in the second because of the lack of suitable nitrogen nutrients. For a thorough bacteriological investigation the starter should be plated out on both these media, since contaminating bacteria, *e.g.* tetracocci, grow better on the former, and yeasts and moulds on the latter.

Since the true starter bacteria do not thrive on these media, it is possible to use a comparatively large quantity of starter for the plating, but not so much that the added milk constituents will enable the lactic acid bacteria to flourish also. 0·01 c.c. can well be used with 10 c.c. of the medium without any such risk, and if there are no contaminating organisms in this quantity the starter may be considered pure. Burri's (18) smear-culture method (Plate I) is particularly well suited for the examination of the purity of a starter.

When the starter is ready for use its titration value, using phenolphthalein as indicator, should be between 90 and 110 c.c. of tenth normal caustic soda solution per 100 c.c. of starter. If the titration is lower it suggests that the bacteria are weakened; if it is higher it suggests infection with stronger acid-producing bacteria or yeasts.

Chemical and bacteriological investigations of starters can only be supplementary to examination of the smell and taste; if these are not what long experience has shown to be correct, the starter cannot be considered satisfactory even if fault can be found with it in no other way.

Influence of the milk on starters.

In order to obtain good results a starter which contains the right mixture of lactic acid bacteria must be used and handled correctly; moreover, the milk used for the cultivation must be of the best possible quality. It is necessary

that it should have no "off-flavour," and be of good quality bacteriologically and normal in all other respects. This is why the milk must be carefully pasteurised before it is inoculated with the starter.

At the same time it is apparent that there are other factors in the milk which play an important part, but these cannot be referred to any definite property nor can a test be suggested for them. It has long been clear that milk from sick animals is not suitable for the preparation of starter, and there is no reason for discussing such milk here since it should not be used in dairies. A point which is of the greatest importance, however, is that milk is occasionally met with which cannot be relied upon to make an ordinary starter (19, 20, 26), but which otherwise appears to be normal and is given by healthy animals.

The greatest variations will naturally be found when the starter is prepared from the milk of single cows, but in certain cases the milk from whole herds shows this deficiency. It seldom happens, it is true, that milk appears to be absolutely useless for starters, but if the milk from various herds is pasteurised, and samples are then inoculated with the same starter and afterwards allowed to stand in the same thermostat to ripen, considerable variations in taste and smell will be found, and a number of them will be obviously poorer than the others. Sometimes a starter is useless the first time it is inoculated into such milk, but usually the deficiency only becomes apparent after two or three inoculations. Whilst in this way different results are obtained by the cultivation of the same starter in different samples of milk, on the other hand if various starters which, though not faulty differ in taste and smell, are cultivated under uniform conditions and in the same milk, no difference will be apparent as a rule after a few inoculations.

The nature of the milk is of decided importance in the production of a good starter. Poor milk often appears to be a little "thinner" than the normal, and starters should be made from milk rich in solids, or, as in large dairies, mixed milk in which the variations are balanced.

It is an old and doubtless correct assumption that the feeding of the cows has an influence on the usefulness of the milk for the making of starters, and milk from cows which have been given good, natural food-stuffs is to be preferred.

At the same time, differences in feeding cannot always explain these results. Thus the author (12) has found that two cows, which had stood in the same stall and received the same food for more than six months, generally yielded milk which differed so much in nature that the one gave outstandingly good starters, whilst the other gave starters completely devoid of aroma. In spite of this the milk was normal chemically, and veterinary examination could detect no disease in the cows either whilst alive or after slaughter.

Since the deficiency in such milk becomes apparent in the lack of aroma in the starter, which acquires a sharp acid taste such as is found in pure cultures of *Streptococcus cremoris*, it may be assumed that the fault is connected with the inability of the betacocci to grow in the milk concerned.

That such is really the case has been shown by examining 100 bacteria from each of two such starters in which there was no difference other than that they had been inoculated into different milk. Whilst 7 of the 100 bacteria from the starter having a good flavour were betacocci, none was found amongst the 100 isolated from the starter with a sharp taste.

Influence of "ripening" on the butter.

Practical experience has, in the course of time, led to the conclusion that there is a close connection between the aroma of the butter and of the starter, and for this reason a good starter is required to have a good and strong aroma.

Comparatively few controlled experiments have been made on the connection between the quality of the butter and of the starter, in spite of the importance of this question. Where such experiments have been made they have confirmed practical experience that the starter with the best flavour gives the best butter, and it should be mentioned that Boekhout and van Beynum (21) found that the ripening of cream with a starter that did not contain betacocci gave a butter that not only was less aromatic, but also had a poorer keeping quality than a corresponding butter produced when an aromatic starter was used.

The ripening of cream also protects the butter from the attack of a number of putrefactive bacteria which may get into the butter from water, but which cannot develop because the reaction is acid. Further, the yield of butter obtained by the churning of ripened cream is slightly higher than that obtained from sweet.

Although ripening the cream can thus protect the butter from certain faults, there are other defects which it promotes. This is particularly true for butter which is to be kept for a very long time and must therefore be held in cold store. In such a case it may be an advantage to omit the ripening, even though the aroma of the butter is not so satisfactory.

CHEESE STARTERS.

The methods of cheese-making have undergone various changes in the course of time, but these only represent a slow development of the old empirical methods of working. It has long been known that cheese is not so good when it is made from perfectly fresh milk as when the milk or part of it has undergone a certain "ripening," and it has gradually become clear that this ripening consists essentially of a development of lactic acid bacteria.

Gradually, as milk was better produced, so that there was not so great a spontaneous bacterial development, these bacteria have been added to the cheese milk, either by means of milk which was already sour, as for example, ordinary buttermilk, or in the form of starter specially prepared for this purpose. The latter method is the more rational and, as with the ripening of cream, should give better control over the bacterial development and yield

a more uniform product. This end is attained to some extent, but since the bacteria work in the cheese for several months, whilst their activity in cream is confined to a day, the souring of milk for cheese-making is much more difficult to control than cream for butter-making.

Hard pressed cheese are not ready for consumption till after long storage. The most pronounced change which occurs during the ripening is the transformation of the proteins whereby the insoluble nitrogen compounds of the fresh cheese are broken down into less complex substances which are soluble in water.

As was pointed out by Freudenreich and Orla-Jensen (22) certain lactic acid bacteria are mainly responsible for this change. According to Orla-Jensen's investigations the splitting of the casein by means of the lactic acid bacteria is brought about by an enzyme which resembles erepsin and which issues from the cells only when they are dead. The importance of the lactic acid bacteria for ripening was first established by studies on Emmental cheese, and a judicious use of bacterial cultures has been of the greatest importance in the making of this cheese. Freudenreich and Orla-Jensen have shown that *Thermobacterium helveticum* (*Bact. casei*) is the chief cause of the particular type of degradation of casein that takes place in Emmental cheese and gives it its characteristic flavour.

Thermobacterium helveticum is found in the stomachs of calves from which cheese rennet is prepared, and the particular methods which have always been used for the preparation of natural rennet in the Emmental cheese factories, combined with the high temperature used in the making of the cheese itself, have secured empirically for those bacteria particularly good conditions for development in the cheese.

In spite of all this it is still uncertain whether these old methods will produce the right bacteria in sufficient numbers, and in Switzerland and those countries in which Emmental cheese is now manufactured, use is often made of cultures of *Tmb. helveticum*, which is sold to the cheese factories by various laboratories as cultures in whey or milk. These cultures are propagated in whey in the factories until a new culture from the laboratory is found to be necessary. Although *Tmb. helveticum* is the most important organism of Emmental cheese, the most recent researches (23) have shown that other bacteria also play a part. These have hitherto entered naturally, but in the factories they are now being used as starters in conjunction with *Tmb. helveticum*.

As a result of its method of production, in Emmental cheese the bacteria that grow at high temperatures are the most prolific, but in the preparation of the more general types of cheese temperatures high enough for the development of *Tmb. helveticum* are not used, and the question arises as to what types of lactic acid bacteria are of special importance for the ripening of these cheese.

The starter which is added to the milk in the preparation of ordinary cheese is exactly the same as that used in the making of butter, and strepto-

cocci will therefore form the bulk of the flora of the fresh cheese; but gradually the streptococci decrease in number whilst *Streptobacterium casei* increases. In ordinary cheese therefore streptococci and streptobacteria are the organisms chiefly found, and whilst the former are added to the milk the latter gradually develop spontaneously.

Since it must be assumed with Orla-Jensen (22) that it is the enzymes liberated from the dead cells which are particularly active in cheese-ripening it may well be thought that the streptococci are the chief agents even though they die very quickly when once the cheese is made.

It has already been mentioned that the various strains of *Streptococcus cremoris* found in starters vary in their powers of splitting casein and it must therefore be assumed that, instead of using the same starter as for butter, a special starter which should contain particularly strong casein-splitting strains of lactic acid bacteria should be prepared for cheese milk (25). Here also an increased effect can be obtained by the interaction of various bacteria, for as Barthel and Sadler (24) have shown, a greater degradation of casein is obtained with the use of a mixed culture such as an ordinary starter, than with single strains isolated from that starter.

There is, however, no doubt that, independently of the streptococci, *Streptobacterium casei* plays a very important part in the ripening of ordinary cheese, since it has a strong casein-splitting power.

Hitherto these bacteria have only been experimentally added as starters. In practice their growth has depended on their accidental introduction into the milk. This doubtless needs to be changed, all the more so since experiments made with the addition of cultures of *Streptobacterium casei* to milk for cheese-making have given very promising results. Even now the use of specially prepared starters for cheese-making is only in the experimental stage, but a great development in this field may be expected, for as cleaner milk is successfully procured the more necessary will it become to add the right bacteria to the cheese milk by means of a special starter.

STARTERS FOR DIETETIC SOUR MILK PRODUCTS.

It is generally agreed that sour milk preparations are excellent foodstuffs, which in many cases are preferred to fresh milk, but there is not sufficient evidence to establish the relative value and the special effects of the various preparations.

In many countries extensive use is made of milk which has been rendered sour either spontaneously or by the use of the same type of starter as that used in butter-making. In some, great value is attached to the use of strains of slime-forming streptococci in the making of sour milk which can be drawn out into long threads.

Kefir has had great renown as a therapeutic substance, and this preparation is constantly used not only in Caucasia, where it originated, but throughout

the world. The bacteriologically interesting symbiosis between *Betabacterium caucasicum* and yeasts, which occurs in kefir, has already been mentioned on page 150 and since it is improbable that there will ever be any suggestion of the preparation of kefir by any other than empirical methods, this product will not be discussed in more detail.

Great interest was awakened when Metchnikoff claimed that yoghurt contained bacteria peculiarly fitted to thrive in the large intestine of man and so prevent the development of the harmful putrefactive bacteria. Since this time yoghurt has been used on a large scale throughout the world. Unlike ordinary sour milk which is made at about 30° C., yoghurt is prepared at 40° C.

If, as Metchnikoff believed, there can be introduced into the human body lactic acid bacteria which will continue to grow in the intestines, they must necessarily grow at blood temperature, and from this point of view yoghurt is to be preferred to ordinary sour milk. *Thermobacterium bulgaricum*, either in pure culture or in combination with other organisms isolated from yoghurt, is now frequently used for the preparation of this product. But at the same time, great doubt is now entertained whether *Thermobacterium bulgaricum* is really capable of living in the human intestine, and in many places in recent years a preference has been shown for the use of sour-milk preparations containing bacteria isolated from the intestines. These are generally termed acidophilus milk, but in reality the bacteria found therein are scarcely identical with the *Bacillus acidophilus* described by Moro, but they seem, like yoghurt bacteria, to be lactic acid bacteria belonging to the genus *Thermobacterium*.

The bacteria found in the acidophilus milk differ from *Tmb. bulgaricum* in growing much more slowly in milk, and many of them will not thrive until a little autolysed yeast, for example, has been added. Since these bacteria sour milk so slowly, sterilised milk must be used for this product, otherwise butyric acid bacteria will readily develop when the milk stands at 37° C., the favourable temperature for acidophilus bacteria.

In conclusion it may be said that the preparation of such sour-milk products does not offer fundamental difficulties, but there is no real understanding of what precisely justifies their reputation. The confidence which has been placed in them has therefore been largely dependent upon the whims of fashion.

BIBLIOGRAPHY.

(1) Orla-Jensen (1919). The lactic-acid bacteria. *Kgl. Danske Vidensk. Selsk., Skrifter Nat. og Math. Afd.* 8 Raekke, V. 2.

(2) Conn (1889–1900). Bacteria in milk, cream and butter and the ripening of cream. *Conn. Agric. Expt. Sta. Ann. Rpts.*

(3) Storch (1890). *Nogle Undersøgelser over Flødens Syrning.* 18 Beretn. fra Forsøgslab.

(4) Weigmann (1890). *Milch-Ztg.* **19**, 593, 944.

(5) Boekhout and de Vries (1919). Aromabildner bei der Rahmsäuerung. *Cbl. f. Bakt.* Abt. 2, **49**, 373.

(6) HAMMER and BAILEY (1919–23). The volatile acid production of starters and of organisms isolated from them. *Iowa Agr. Exp. Sta. Research Bull.* 55, 63, 65, 66, 67, 80, 81.

(7) STORCH (1919). *Fortsatte Undersøgelser over Fremstillingen af Syrevaekkere.* 102 Beretn. fra Forsøgslab.

(8) HENNEBERG (1926). *Handbuch der Gärungsbakteriologie.* 2 Aufl. 1, 439.

(9) VAN DAM (1918). Über den Einfluss der Milchsäure auf die Milchsäuregärung. *Biochem. Z.* 87, 107.

(10) SVANBERG (1919). *Über einige milchsäurebakteriologische pH Bestimmungen.* Inaug.-Diss. Stockholm.

(11) DREWES (1927). Über die Bactericidie der Milch. *Milchw. Forsch.* 4, 409.

(12) KNUDSEN AND SØRENSEN (1929). Bidrag til Syrevaekkernes Bakteriologi. *Kgl. Vet. og Landbohøjskole Aarskr.* 64.

(13) HAMMER and SHERWOOD (1923). The volatile acids produced by starters, etc. *Iowa Agr. Exp. Sta. Research Bull.* 80.

(14) AYERS and MUDGE (1921). Two organisms of a commercial lactic starter. *J. Dairy Sci.* 4, 241.

(15) S. ORLA-JENSEN, A. D. ORLA-JENSEN and SPUR (1926). The butter aroma bacteria. *J. Bact.* 12, 333.

(16) VAN NIEL, KLUYVER and DERX (1929). Über das Butteraroma. *Biochem. Z.* 210, 234.

(17) SCHMALFUSS and BARTMEYER (1928). Diacetyl ein Stoffwechselprodukt? *Z. f. physiol. Chem.* 176, 282.

(18) BURRI (1928). The quantitative smear-culture. *Report of World's Dairy Congress,* p. 690.

(19) BAKER and HAMMER (1926). Influence of the milk on the starter made from it. *Iowa Agr. Exp. Sta. Research Bull.* 92.

(20) KNUDSEN (1926). Undersøgelser over Syrevaekkere. *Maelkeritidende,* p. 235.

(21) BOEKHOUT and VAN BEYNUM (1926). Aromabacterien en botergebreken. *Vereeniging tot exploitatie eener proefzuivelboerderij te Hoorn,* Verslag over het jaar 1926, p. 266.

(22) ORLA-JENSEN (1921). *Dairy bacteriology.* London: Churchill.

(23) —— (1930). Tätigkeitsbericht der eidgenössischen milchwirtschaftlichen und bakteriologischen Anstalt, Liebefeld-Bern. *Landwirtschaftl. Jahrbuch d. Schweiz,* p. 192.

(24) BARTHEL and SADLER (1928). The casein-splitting properties of starter. *Report of the World's Dairy Congress,* p. 654.

(25) LEITCH (1924). De quelques progrès récents dans la fabrication du beurre. *Le Lait,* 4, 732.

(26) KELLY (1930). Is all milk equally suitable as a medium for the preparation of starters? *Scientific Agriculture,* 10, 328.

(*MS. received for publication,* 26. 2. 31.)

19. THE INFLUENCE OF OTHER BACTERIA ON THE PRODUCTION OF ACID BY LACTIC STREPTOCOCCI IN MILK

By G. A. COX and H. R. WHITEHEAD.

Dairy Research Institute (N.Z.), Massey Agricultural College, University of New Zealand.

(With 9 Figures.)

The effects produced by the growth of a mixed bacterial flora, as distinct from the effects produced by pure cultures, are of the utmost importance in dairy bacteriology. Milk usually contains a variety of bacteria, and the changes which it undergoes are, therefore, the result of the associative growth of several species. Comparatively little is known of the influence of one organism upon another during growth in milk, yet, particularly in cheese manufacture, such information would be valuable. The effects of other organisms present in milk on the growth and activities of lactic streptococci probably provide an explanation for cases of abnormality in the progress of acid production in the cheese vat.

In recent years a considerable amount of work has been reported on the effects of bacteria on one another in associative growth, and it has become evident that there are two main classes of phenomena:

(*a*) a simple stimulating or inhibiting action of one organism upon another;

(*b*) a modification of the metabolism of one or both of two organisms in a mixed culture.

The antagonistic action of bacilli of the aciduric group (*e.g. B. acidophilus*) against other organisms is an example of the first type of action. It has been shown that *B. acidophilus* will inhibit and even destroy putrefactive anaerobes, *B. coli*, and many strains of streptococci (Schiller(7)). On the other hand, Rogers(6) has shown that lactic streptococci inhibit the growth of *B. bulgaricus*. In some instances it has been shown that bacteria can metabolise bacterial protein (Gratia and Rhodes(2)), and thus manifest a "cannibalistic" action.

There are many examples of the second type of action. As early as 1892, Nencki(3) showed that a mixed culture of *B. paralactici* and *B. chauvoei* will produce glucose from normal butyl alcohol, a reaction which is not brought about by either organism growing alone. More recently Castellani(1) has demonstrated a similar phenomenon in connection with gas production by bacteria. He has shown that a mixture of two organisms may ferment certain carbohydrates with the formation of gas, whereas neither organism in pure culture will produce any gas. On the other hand, Smith and Smith(8) have observed that *B. coli* sometimes loses its property of gas production when

grown in association with other Gram-negative bacilli. All of these results have yet to receive their full interpretation; and there are many conflicting observations which cannot at present be reconciled. Much more detailed investigation of the physiology of bacteria is needed before it will be possible to predict the results to be expected from the growth of a mixed flora in a given medium.

An essential part of the process of cheese manufacture is the formation of lactic acid by the metabolic activity of lactic streptococci in the milk and curd. Milk delivered to cheese factories always contains what may be called "contaminant organisms." Since difficulties in manufacture are often experienced owing to delay or cessation of acid production, it is of importance to know what effects such organisms exert on the growth and metabolism of the streptococci. It has been suggested that certain organisms will inhibit the growth of streptococci in milk or will produce alkali sufficient to neutralise the lactic acid produced (Morgan and Curle (5)), but conclusive evidence has yet to be adduced.

The experiments described below were intended mainly as a preliminary study of the effects of several organisms on the growth and metabolism of lactic streptococci in milk as indicated by rate of acid production. Four organisms commonly found in contaminated milk were used, namely, *B. coli communior*, *B. faecalis alkaligenes*, a staphylococcus and a sporing bacillus of the *subtilis* group. The general scheme of the experiments was to determine the acidity produced in a given time by the growth of each organism in sterile separated milk at 30° C., and the acidity produced by mixtures of the contaminant organisms with lactic streptococci. Thus, from the results, it was possible to decide whether the organisms exerted any influence on one another in mixed cultures, so far as acidity production was concerned.

At the beginning of the investigation some difficulty was experienced in devising a suitable technique for measuring equal amounts of inoculum, particularly in the case of lactic streptococci. The original procedure was to dilute a coagulated milk culture of lactic streptococci with sterile saline or water, and, after thorough agitation, to distribute the suspension with a sterile pipette into flasks of sterile separated milk. When this technique was used, flasks inoculated with equal volumes of suspension sometimes gave widely divergent acidity results after incubation. Eventually the difficulty was overcome by the use of the following technique.

The streptococci were grown on yeast whey agar, and the growth produced in 24 hours at 30° C. was washed from the surface of the agar with sterile water. The suspension so obtained was shaken vigorously. Samples of such a suspension inoculated into flasks of milk and incubated for some time gave reasonably concordant duplicate acidity titrations. Most probably the streptococci are unevenly distributed in a diluted milk culture because some organisms are entrapped in clumps of coagulated casein which subsequently redissolve in the separated milk into which the suspension is inoculated. The following table gives typical results obtained with the original method and

with the method that was finally adopted throughout the remainder of the experiments. The table serves further to show the order of accuracy of results obtained by the method used.

Table I.

Original method		Final Method	
	Increase in acidity in flasks inoculated with the same volume of diluted milk culture c.c. of *N*/10 NaOH per 10 g. culture		Increase in acidity in flasks inoculated with the same volume of suspension washed from yeast whey agar c.c. of *N*/10 NaOH per 10 g. culture
Exp. 1.	3·0	Exp. 3.	2·55
	1·95		2·65
	4·80		2·65
	3·10		2·80
	4·80		2·70
	5·80		2·70
Exp. 2.	3·20		
	1·50		
	2·95		
	1·55		

Associative growth of *Strep. lactis* and *B. subtilis*.

Experimental.

The organisms were pure cultures taken from single colonies. Both organisms were grown on yeast whey agar for 24 hours at 30° C. The growth was washed from the surface of the agar with sterile water and transferred to flasks containing about 500 c.c. of sterile water. The flasks were fitted with sterile rubber stoppers, and vigorously shaken in order to break down clumps and provide a fine even suspension of bacteria. Approximately the same amount of growth of each organism was used in the different experiments, but no further attempt was made to standardise the suspension.

Flasks containing exactly 200 c.c. of separated milk of good sanitary quality were steamed for 30 minutes and allowed to cool. Duplicate flasks were then inoculated with the suspensions of the two organisms, according to the following table. All of the flasks were finally adjusted to the same volume by addition of sterile water.

Table II.

Flask No.		Flask No.	
1. 2.	10 c.c. *Strep.* suspension	7. 8.	10 c.c. *Subtilis* suspension
3. 4.	20 c.c. *Strep.* suspension	9. 10.	20 c.c. *Subtilis* suspension
5. 6.	30 c.c. *Strep.* suspension	11. 12.	30 c.c. *Subtilis* suspension

Flask No.		
13. 14.	10 c.c. *Strep.*;	10 c.c. *Subtilis*
15. 16.	10 c.c. *Strep.*;	20 c.c. *Subtilis*
17. 18.	10 c.c. *Strep.*;	30 c.c. *Subtilis*
19. 20.	20 c.c. *Strep.*;	10 c.c. *Subtilis*
21. 22.	30 c.c. *Strep.*;	10 c.c. *Subtilis*

An initial titration was carried out on a few of the milk samples and the flasks were then incubated at 30° C. for 9 to 11 hours (depending on the rate of acid development in each experiment). After the lapse of this time, 1 c.c. of 1 per cent. $HgCl_2$ was added to every flask to check the growth in all flasks simultaneously, and 10 g. of milk from each flask was titrated with N/10 NaOH, using phenolphthalein as indicator. The duplicates always gave good agreement to the extent indicated in Table I. The increases in acidity were plotted on two graphs. On one graph were plotted the results from those flasks in which the smallest amount of *B. subtilis* was present in association with varying amounts of *Strep. lactis*, and on the other, the results from those flasks in which the smallest amount of *Strep. lactis* was present in association with varying amounts of *B. subtilis*. In addition, curves were drawn on each graph showing the increases in acidity produced by varying amounts of the organisms growing in pure culture, and the theoretical acidity increases assuming the effects of the two organisms to be additive. Thus, on the first graph (Fig. 1), curve *A* shows the acidity increases given by different amounts of *Strep. lactis* suspension (flasks (1, 2), (3, 4), and (5, 6)). Curve *B* shows the theoretical acidity increases given by the different amounts of *Strep. lactis* suspension, plus the smallest amount of *B. subtilis* suspension, on the assumption that the effects are additive; curve *C* shows the actual acidity increase by the same mixtures (flasks (13, 14), (19, 20), and (21, 22)). The same explanation applies to the second graph (Fig. 2) if the names of the organisms are transposed. In spite of the fact that the actual numbers of each type of organism varied in each experiment owing to the very rough method used for standardisation of the suspensions, the curves plotted from the results of five experiments carried out on the above plan were very similar in shape. Figs. 1 and 2 are typical graphs (prepared from the averages of the figures) and illustrate the general results to be expected in experiments with these two organisms.

It is evident from the graphs that associative growth of *Strep. lactis* and *B. subtilis*, under the experimental conditions described, gives an increase in acidity production greater than would be expected if the effects of the growth of the two organisms were simply additive. The stimulating effect rapidly increases as the amount of *B. subtilis* inoculum is increased. With a constant amount of *B. subtilis* there is apparently a definite amount of *Strep. lactis* which produces the maximum effect (maximum point on curve *C*, Fig. 1). This was evident in all of the experiments. At first sight this appears to indicate that a maximum acidity increase is produced when the organisms are mixed in a certain proportion. However, it was not possible to standardise accurately the number of organisms in the bacterial suspensions, and the numbers varied in each experiment. The factor determining the position of the maximum on curve *C*, Fig. 1, therefore, cannot be connected with the proportion between the numbers of the two organisms; and it appears more likely that it is connected with the particular stage reached by the growth of the lactic streptococci and the acidity of the medium. It will be observed

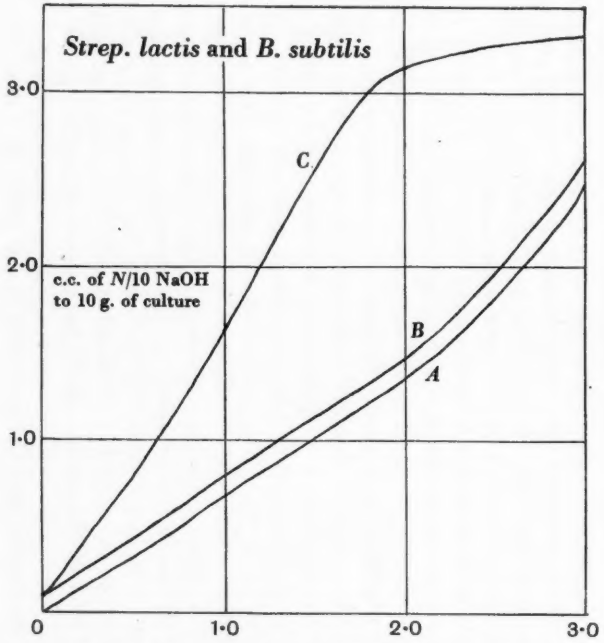


Fig. 1. Parts of *Strep. lactis* suspension to one part of *B. subtilis* suspension.

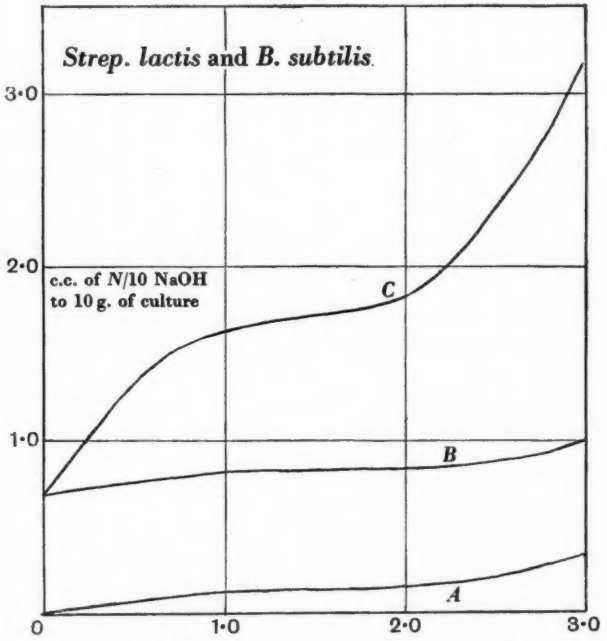


Fig. 2. Parts of *B. subtilis* suspension to one part of *Strep. lactis* suspension.

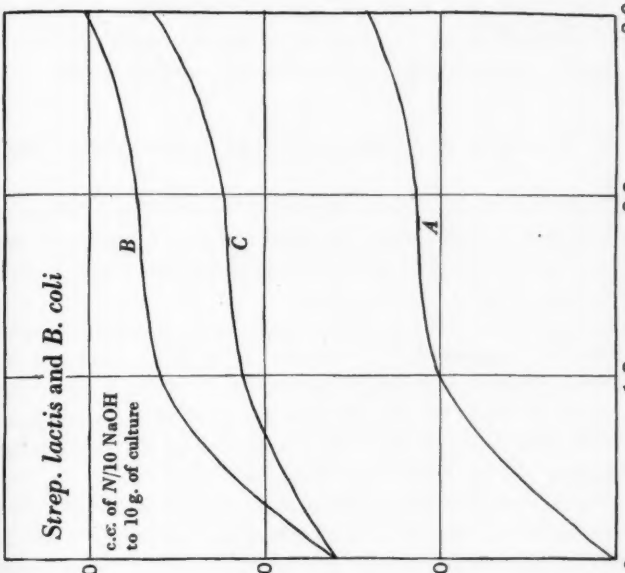


Fig. 4. Parts of *B. coli* suspension to one part of *Strep. lactis* suspension.

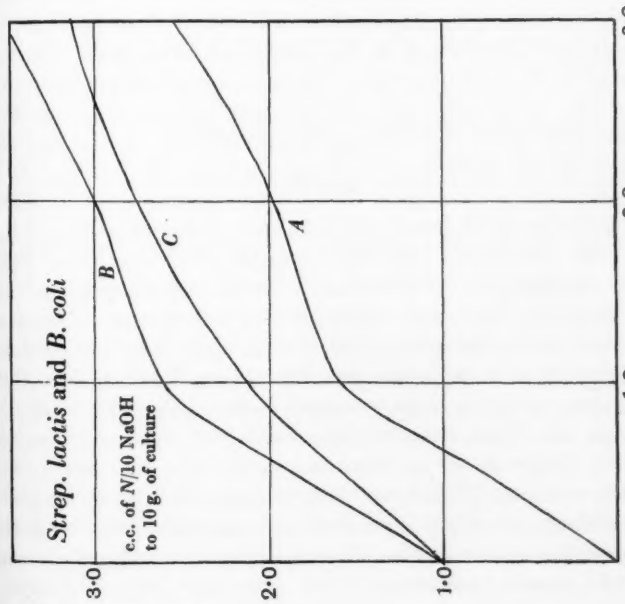


Fig. 3. Parts of *Strep. lactis* suspension to one part of *B. coli* suspension.

that whatever the amounts of the two organisms, there is always a stimulation of acid production, and, in general, this is in agreement with the results of the work of Marshall (4).

Associative growth of *Strep. lactis* and *B. coli communior*.

The experimental details in these experiments were exactly the same as those described above. Here, again, the results of four experiments were in good agreement; and it is most convenient to summarise the results in the form of curves, which are typical of all the results.

These graphs indicate that there was a fairly uniform effect whatever the amounts of the two organisms. The association of *Strep. lactis* and *B. coli* evidently causes a depression in acidity production from the value that would be expected if the effects of the two organisms were additive. In no instance, however, did the addition of *B. coli* cause the acidity of the mixed culture to be depressed below the value given by *Strep. lactis* alone.

The experiments with *B. coli* and *Strep. lactis* were repeated with the same cultures some three months later, with somewhat surprising results. The graph below (Fig. 5) illustrates the average results obtained in two experiments carried out during the same week. In these experiments the amount of *B. coli* suspension was not varied.

It will be observed that in these experiments there was a marked stimulation of acid production on the addition of *B. coli* to *Strep. lactis*, curve *C* almost resembling that obtained for *B. subtilis* and *Strep. lactis*. The reason for the change in the type of result is not clear, but there are two possible explanations. In the first place the culture of *Strep. lactis* had lost a great deal of its vitality, as is indicated by its lower acid production and the fact that a few days after its use in these latter experiments it ceased to clot milk in 24 hours at 30° C. If this change in vitality were responsible for the different results one would expect different effects due to associative growth of *B. coli* and *Strep. lactis* according to the vitality of the latter organism. Further observations on this point are necessary. On the other hand, the change in vitality may have been more apparent than real, for these experiments were carried out during the autumn, when most of the cows from which the milk was derived were in the later stages of lactation. It was noticed that all of the stock strains of *Strep. lactis* grew more slowly and produced less acid at this particular time. Thus, the effect observed in the experiments may have been due to a change in the medium used rather than to a change in the vitality of the organisms. Whatever be the real reason, it is evident that the possibility of these variations in medium and organisms must be borne in mind in interpreting results, and in applying such interpretation to phenomena observed under practical conditions.

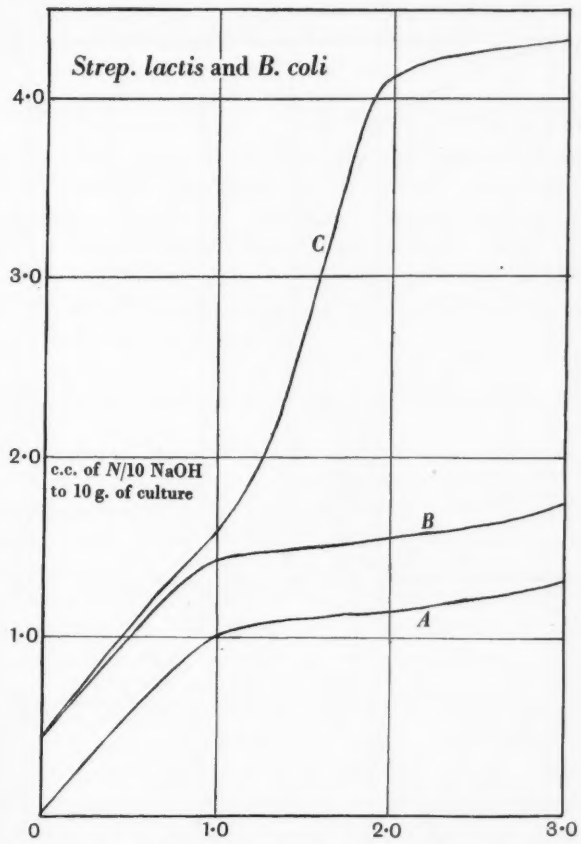


Fig. 5. Parts of *Strep. lactis* suspension to one part of *B. coli* suspension.

Associative growth of *Strep. lactis* and staphylococci.

In all three experiments carried out with these two organisms, the effects due to associative growth were much smaller and were somewhat more variable in the different experiments. Figs. 6 and 7 show with reasonable accuracy, however, the results to be expected in experiments on these lines. The staphylococcus in pure culture produced no measurable acid or alkali within 12 hours, in milk, so that curve *A* falls on the base line in one of the graphs and is coincident with curve *B* in the other graph.

From the curves it is evident that the only significant effect of associative growth is an unexpected increase in acid production when either organism is present in large quantity, together with a small amount of the other organism. From a practical view point, therefore, the only reaction of importance

Fig. 7. Parts of *Staphylococcus* suspension to one part of *Strep. lactis* suspension.

Fig. 6. Parts of *Strep. lactis* suspension to one part of *Staphylococcus* suspension.

would be a slight stimulation of acid production in cultures of lactic streptococci, when contamination with staphylococci occurred.

Associative growth of *Strep. lactis* and *B. faecalis alkaligenes*.

The results of six experiments carried out with these two organisms gave quite consistent results. The graphs below illustrate the normal results in these experiments.

It will be observed that, although the production of alkali by *B. faecalis alkaligenes* in some instances results in a slight depression in the acidity of mixed cultures, yet with larger amounts of either organism there is a tendency towards stimulation of acid production.

Discussion.

From the results so far obtained, it is impossible to say whether the several effects are due to the influence of one organism on the rate of growth of another, or to modifications in metabolism due to associative growth. In order to determine the actual mechanism, it would be necessary to determine the number of organisms present in the different cultures. So far, the experiments give no indication that any of the common "contaminant" organisms in milk will cause a depression in the acid production of lactic streptococci sufficient to account for the abnormal cessation of acid development sometimes encountered in milk and cheese curd. In this connection, however, the experimental conditions must be taken into account. The medium used was milk which had been heated to boiling point for half-an-hour. This treatment may have produced changes which would modify the growth of the bacteria. Furthermore, the titrations were carried out only at one time during the growth of the organisms, and it is possible that at some other stage of growth there may be quite different effects. The titrations, however, were taken during the most active growth phase; and it is precisely during this phase that an abnormal cessation of acid development is sometimes experienced under practical conditions. Again, the experimental conditions did not simulate those in cheese curd, where the organisms are growing in a semi-solid medium. One would expect, however, a quantitative rather than a qualitative difference in effect due to changed conditions. In the authors' opinion, the most important difference between the conditions in the experiments and those occurring in cheese practice is that in the experiments the contaminant organisms were added to the milk at the same moment as the lactic streptococci, whereas, normally, the lactic streptococci are added to milk in which contaminant organisms have been growing for several hours, at least. It seems not unlikely that different effects might be observed under the latter set of conditions, due either to the firmer establishment of the contaminant organisms in the medium, or to the production of toxic substances during their growth. The work is

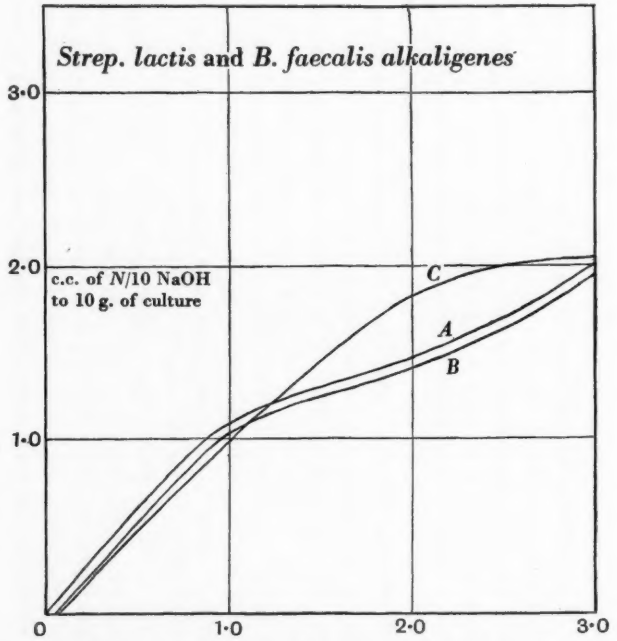


Fig. 8. Parts of *Strep. lactis* suspension to one part of *B. faecalis alkaligenes* suspension.

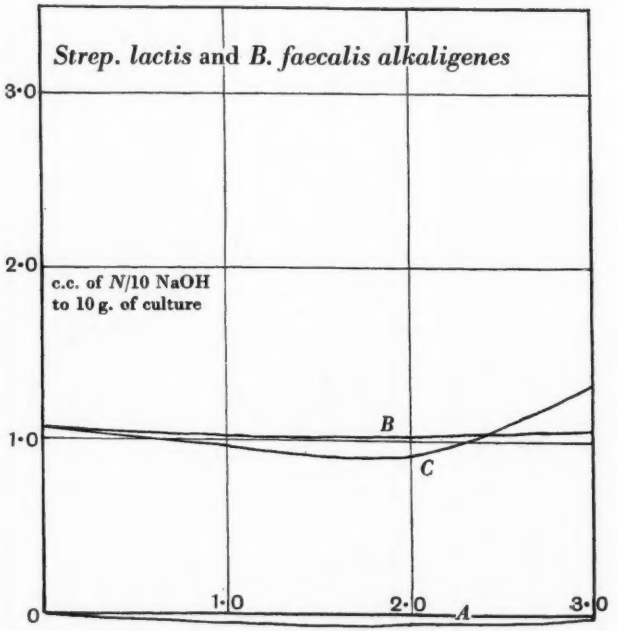


Fig. 9. Parts of *B. faecalis alkaligenes* suspension to one part of *Strep. lactis* suspension.

being advanced along these lines, and indications have already been obtained that different results are to be expected when there is an interval between the inoculation of one organism and another.

Summary.

Four organisms commonly found in contaminated milk (*B. coli*, *B. subtilis*, a staphylococcus, and *B. faecalis alkaligenes*) were grown in milk in association with lactic streptococci, and the acidity produced in a given time was determined. *B. subtilis* appeared to stimulate the production of acid. *B. coli* caused a decrease in acid production in some experiments, and an increase in others. The staphylococcus had a slight stimulative effect. *B. faecalis alkaligenes* had only a slight effect which was barely beyond experimental error. There was no indication that any of the organisms are responsible for the cessation of acidity production which sometimes occurs in milk during cheese manufacture. However, the results were not conclusive in this connection, since the experimental conditions did not simulate those obtaining in a cheese vat.

REFERENCES.

(1) Castellani (1925). *Brit. Med. J.* **2**, 734.
(2) Gratia and Rhodes (1924). *Compt. Rend. Soc. Biol.* **90**, 640.
(3) Nencki (1892). *Cent. f. Bakt.* I. Orig. **11**, 225.
(4) Marshall (1920). *J. Dairy Science*, **3**, 406.
(5) Morgan and Curle (1928). *New Zealand J. Agric.* **37**, 305.
(6) Rogers (1928). *J. Bact.* **16**, 287.
(7) Schiller (1914). *Cent. f. Bakt.* I. Orig. **73**, 123.
(8) Smith and Smith (1920). *J. Gen. Physiol.* **3**, 21.

(*MS. received for publication*, 9. 10. 30.)

20. PASTEURISED MILK FOR CHEDDAR CHEESE MAKING. II

THE MINERAL CONTENT OF CHEDDAR CHEESE

By GEORGE M. MOIR, M.Sc., A.I.C., Ph.D.

National Institute for Research in Dairying, University of Reading.

The preceding investigation(1) left a little doubt as to the effect produced by pasteurisation of clean milk upon the flavour of the mature cheese. For although the cheese made from milk, flash-pasteurised at 165° F., appeared to develop a desirable flavour more rapidly than the raw control throughout the greater part of the ripening period, yet at the end this was spoilt by a very slight bitterness.

The opportunity was therefore taken of having a small series of cheese made, so that one of each of a number of pairs was made from heated milk and the other from unheated. The pasteurisation of the milk was carried out in the case of the first three by heating in the jacketed cheese vat for half-an-hour at 145° F., while the last three were heated in the same way rapidly to 160° F. and immediately cooled. The highest temperature reached by the milk in the case of No. 4 was 165° F. In this also the times taken to heat and cool were rather prolonged. As a result of this excessive heating the resulting cheese developed blue mould to a considerable extent. There was also a little blue mould in No. 5.

The whey drawn from the different vats was filtered through a close-textured cloth. That from the raw milk filtered readily and left practically nothing upon the cloth but a few tiny particles of white curd. But filtration of the whey from the pasteurised milk, especially that heated to the higher temperatures, usually took place slowly and upon the cloth was left a colourless, rather gelatinous layer quite distinct from the white curd particles. Similar material (probably denatured albumin) was obtained in a previous experiment(1).

The cheese were examined by a competent judge when six months' old and, taking them altogether, very little difference was found between the raw and pasteurised of each pair. Whatever slight differences were observed were rather in favour of the cheese from the unheated milk. In the case of Nos. 4 and 5, where blue mould had developed in the cheese from the heated milk, the basis for comparison was of course disturbed, but these cheese possessed a flavour satisfactory for the mould-ripened type.

Although the experiment was planned with the object of obtaining preliminary qualitative information as a guide for future investigations, it was decided, after the cheese had been examined, to determine the *p*H of each,

since this could rapidly be done with the quinhydrone electrode according to the method of Knudsen (2). The results obtained (Table I) showed that in each case the cheese from the pasteurised milk had a lower *p*H than that from the raw milk, except where blue mould had developed. It was suspected at once that this difference might be due to different mineral content of the cheese, for in the previous experiments differences had been found in the mineral content of the whey of cheese from raw and from pasteurised milk. These differences were attributed to the fact that in the pasteurised milk vats the acidity of the whey tended to develop more rapidly than in the raw. The increasing acidity would dissolve mineral material from the curd and it is to be expected that in order to neutralise the acid, basic substances (lime) would thus be dissolved more readily than acidic ones (phosphates). Thus the resulting

Table I. *Analyses of cheese. Acidities as per cent. lactic acid.*

Cheese No.	% of Ca	% of P	Ratio Ca/P	*p*H	Milk before rennet	Whey at cutting	Whey after drawing	Whey from press
Raw 1	—	—	—	5·17	0·20	0·16	0·35	0·84
Past. 1	—	—	—	5·12	0·195	0·19	0·38	0·84
Raw 2	—	—	—	5·33	0·19	0·14	0·24	0·17
Past. 2	—	—	—	5·11	0·19	0·155	0·30	1·14
Raw 3	0·830	0·560	1·48	5·44	—	0·15	0·20	1·0
,,	0·828	0·563	1·47					
Past. 3	0·667	0·549	1·21	5·07	—	0·155	0·40	1·15
,,	0·668	0·558	1·20					
Raw 4	0·821	0·593	1·39	5·26	0·20	0·15	0·31	1·18
,,	0·814	0·579	1·43					
Past. 4	0·431	0·433	0·996	6·69	0·22	0·19	0·41	1·19
,,	0·437	0·438	0·997					
Raw 5	0·682	0·521	1·31	5·17	0·21	0·165	0·31	1·01
,,	0·691	0·531	1·30					
Past. 5	0·546	0·477	1·15	5·95	0·205	0·18	0·47	—
,,	0·531	0·465	1·14					
Raw 6	0·786	0·575	1·37	5·38	0·205	0·155	0·21	0·95
,,	0·791	0·573	1·38					
Past. 6	0·753	0·575	1·31	5·02	0·20	0·15	0·32	0·88
,,	0·746	0·565	1·32					

cheese from the pasteurised milk ought to have a lower proportion of calcium to phosphorus than that from the raw milk. When analyses were carried out (by the methods described in the previous paper (1)) this expectation was fulfilled, even in the cheese where subsequent development of mould had altered the *p*H distinctly to the alkaline side of what is usual in Cheddar cheese, *p*H 5·0–5·5.

The acidity figures (obtained from the cheese-maker's record) show that although the two types of milk were usually rennetted at the same acidity yet in the vat of pasteurised milk the acidity increased very much more rapidly until after the whey was drawn. Between this stage and the pressing the differences were reduced as a result of modifications in the treatment of the curd by the cheese-maker. In the case of No. 4, where the greatest difference exists between the Ca : P ratio of the raw and the pasteurised, the vat of pasteurised milk was rennetted at a distinctly higher acidity than the raw.

On the other hand in No. 6 where this ratio shows the smallest difference the acidityof the raw vat was higher at rennetting, cutting and from the press. The pasteurised vat, however, between cutting the curd and removing the whey developed sufficient acidity to produce the slight differences obtained.

Apart from investigation related to dietetics (3) the mineral content of Cheddar cheese has not received much attention. Williams (4) showed that American Cheddar cheese obtained from diverse sources contained minerals which were present so that the ratio $CaO : P_2O_5$ differed little from 1, which is equivalent to a Ca : P ratio of 1·64—a somewhat higher figure than has been obtained in the present analyses. Van Slyke and Bosworth (5) carried out a very interesting series of experiments upon the changes which take place in the solubility of the calcium and the phosphorus present in the cheese during ripening, but the tendency in modern investigations of the chemical changes during cheese-ripening has been to consider only the changes in the nitrogenous compounds.

It is clear that pasteurisation modifies the milk so that the lactic acid flora is able to develop much more rapidly. One of the results of this altered development is the production of a cheese possessing a different mineral content and consequently a different *p*H. Whether these differences are important in modifying the course of the ripening is a matter for future investigation, but it is well known that bacterial metabolism is capable of being considerably affected by changes in *p*H.

The writer wishes to acknowledge the assistance of Miss D. V. Dearden who made and judged the cheese for this investigation. The acidity figures of Table I have also been obtained from her records.

REFERENCES.

(1) Moir (1930). *J. Dairy Res.* **1**, 149.
(2) Knudsen (1925). *Zeit. Unt. d. Nahr. u. Genuss.* **50**, 300.
(3) Blunt and Summer (1929). *Chem. Abstr.* **23**, 448.
(4) Williams (1927). *J. Ass. Off. Agric. Chem.* **10**, 302.
(5) van Slyke and Bosworth (1907). *New York Agric. Exp. Sta. Tech. Bull.* **4**.

(*MS. received for publication*, 5. 7. 30.)

21. THE INFLUENCE OF THE STAGE OF LACTATION ON THE NATURAL ACIDITY OF MILK

By J. HOUSTON, M.Sc.

Chemical Research Division, Ministry of Agriculture (N.I.), Queen's University, Belfast.

(With 1 Figure.)

THE natural acidity of milk is known to vary from one individual cow to another. Variations are also found in the course of a milking and even from one quarter of the udder to another of the same cow. It is therefore of practical as well as of scientific interest to study the various factors which influence acidity and to see how the properties of milk are affected by them.

This investigation was undertaken with a view to providing information on how natural acidity varied, particularly with the advance of lactation.

THE CAUSE OF ACIDITY IN MILK.

The natural acidity of milk is generally attributed to one or all of the following substances: monobasic phosphates, caseinogen, acid citrates and carbon dioxide. Rice and Markley (1) show that carbon dioxide, caseinogen, citric acid and albumin account for about half of the acidity. The difference between the total acidity and that due to the factors enumerated they attribute to phosphates. They show also that the difference in acidity between the various samples is due to phosphates, and advance the hypothesis that a condition of equilibrium exists between certain of the milk constituents, particularly citric acid, phosphoric acid, caseinogen and the bases. They state that it is this equilibrium which determines the natural acidity of milk and that the relative proportions of each constituent will modify both the equilibrium and the acidity. On the other hand, Tocher (2) states that the acidity of freshly drawn milk depends on (*a*) the percentage of lactose present, (*b*) the number of lactose fermenters in the milk and (*c*) the interval between milking and the time of making the test. He concludes, therefore, that natural acidity is due to the slight decomposition of lactose between the time of milking and the time of making the test. This apparent divergence of opinion may be explained partly by the fact that Tocher's tests were made on samples at least three hours old. The term "natural acidity" in the literature usually refers to the reaction of milk tested immediately the milking operation is completed, therefore natural acidity cannot be due to any extent to the development of lactic acid.

THE VARIATION IN THE ACIDITY OF MILK.

Factors which may influence the acidity as well as the composition of milk are—health of cow, feeding, age, breed, lactation, differences between morning and evening milk, different portions of the same milking, different quarters of

the udder, and the composition of the milk. Rice and Markley (1) show that variations may range from 0·086 per cent. to 0·229 per cent. calculated as lactic acid, and that high acidity in milk is associated with a high phosphate content. Duncombe (3) shows that there is little if any effect on the reaction of milk from the feeding of lactic, butyric, acetic and phosphoric acids, but noted a marked decrease in total acidity when the cows were changed from winter to summer rations. Mattick and Hallett (4), who examined the milk from each quarter of one cow during a lactation, found that the milk from all quarters decreased in acidity as lactation advanced. They found also that there were significant differences between the acidities of milk from the same quarters at successive milkings and also between the acidities of milk from different quarters.

Details of investigation.

This investigation was carried out, as part of a more detailed investigation of the causes of variations in the composition of milk, on the herd of crossbred Shorthorn cows at the Agricultural Research Institute of Northern Ireland, Hillsborough, Co. Down, between February 1929 and November 1930. This herd is licensed to produce Grade A (T.T.) milk, and is housed in a modern byre, one half of which is lighted with Vita glass. Nutritional experiments, relating to mineral requirements, are in progress, and half the cows are receiving a mineral supplement to their ration. Thus the herd is divided into four feeding groups, two of which are given additional minerals; one mineral group and one non-mineral group are housed in that half of the byre which is lighted with Vita glass. During the investigation the number of cows in milk in the herd varied from twenty-five to thirty-three. The average number of cows in a feeding group was between seven and eight.

The cows were fed according to milk yield. In winter, all cows received hay, 30 lb. of roots, and a meal mixture consisting of:

	Parts by weight
Soya bean meal	1
Earth nut cake	1
Maize gluten feed	2
Maize meal	2
Crushed oats	2
Bran	1

In the rations of the lower-yielding cows, part of the hay was replaced by oat straw. In summer, the higher-yielding cows received a supplementary ration. The mineral supplement[1] during the experiment was fed at the rate of 3½ lb. per cwt. of concentrates, and consisted of:

	Parts by weight
Steamed boneflour	10·0
Salt	1·5
Potassium chloride	2·0
Sulphur	0·25
Iron oxide	0·1
Potassium iodide	0·01

[1] This mineral supplement has now been modified, and in addition to the constituents mentioned, contains calcium carbonate.

During the winter the cows were turned out for two to four hours daily, when the weather was suitable. Milking commenced at 6.30 a.m. and 4.30 p.m.

Throughout the investigation detailed particulars were kept in the form of a diary of dates of calving, service, oestrum and illness, as well as information relating to weather conditions, milk yields, butterfat and solids-not-fat yields, milkers, times of milking, etc. All acidity data are based on weekly tests made on each cow's evening milk immediately after milking and before the milk had cooled. Acidity was determined by titrating 10 c.c. of the sample with $N/9$ soda, using phenolphthalein as indicator. Results are in all cases expressed as the number of cubic centimetres of $N/9$ soda required to neutralise 10 c.c. of milk.

Discussion of results.

Over 2500 tests were made and results obtained for 39 complete and 38 incomplete lactations. The average of all the tests was 1·89 c.c. $N/9$ and of the complete lactations 1·90 c.c. $N/9$. The average acidities of individual lactations ranged from 2·35 c.c. $N/9$ to 1·54 c.c. $N/9$. The acidities of individual milkings of all cows ranged from 2·70 c.c. $N/9$ to 1·05 c.c. $N/9$. The greatest difference in the acidity of individual milkings of a cow during a lactation was 1·45 c.c. $N/9$. The tests for this cow ranged from 2·50 c.c. $N/9$ at the beginning of the lactation to 1·05 c.c. $N/9$ when the cow was almost dry. If this latter test be disregarded the next lowest test for this cow is 1·70 c.c. $N/9$ which gives a range of 0·80 c.c. $N/9$. A more extreme range of acidity over a lactation has been noted by Mattick and Hallett (4). The acidity of the milk of the cow tested by these workers varied from 2·91 c.c. $N/9$ at the beginning of the lactation to 1·07 c.c. $N/9$ at the end, a difference of 1·84 c.c. $N/9$. The former test, however, refers to morning milk and the latter to evening milk, so that the results are not quite comparable. The tests of the cow showing the least variation in acidity over a lactation ranged from 1·90 c.c. $N/9$ to 1·55 c.c. $N/9$, a difference of 0·39 c.c. $N/9$.

In order to compare the acidity tests for lactations of varying duration and to arrive at a curve which would show the variation in acidity during a lactation, it is necessary to allow for the length of lactation. Since in the middle of the lactation the acidity tests do not vary greatly, and since it is at the beginning and end of the lactation that changes occur, the data were tabulated and averaged twice, firstly for the first eighteen weeks of all lactations, and secondly for the last eighteen weeks (Table I). This accounts for the break in the middle of the curve (Fig. 1). Where a lactation was of less than forty weeks' duration, the tests were divided equally between the two halves of the curve.

The data in Table I represent the averages of the thirty-four complete lactations. Lactations of less than thirty weeks' duration were omitted. The standard deviations of the averages are included to show the significance of the variations. The total range of the average acidities of the thirty-four lactations is from 2·27 c.c. $N/9$ to 1·65 c.c. $N/9$, a difference of 0·62 c.c. $N/9$.

Table I. *The average acidity of milk during a lactation.*

Weeks in milk	Average acidity c.c. $N/9 \pm$ S.D.	Weeks from end of lactation	Average acidity c.c. $N/9 \pm$ S.D.
1	2·27 ± ·047	18	1·89 ± ·052
2	2·14 ± ·034	17	1·90 ± ·049
3	2·03 ± ·043	16	1·90 ± ·038
4	2·00 ± ·038	15	1·88 ± ·044
5	1·96 ± ·031	14	1·85 ± ·042
6	1·94 ± ·036	13	1·83 ± ·045
7	1·93 ± ·041	12	1·86 ± ·035
8	1·90 ± ·042	11	1·88 ± ·043
9	1·92 ± ·035	10	1·86 ± ·042
10	1·91 ± ·043	9	1·85 ± ·040
11	1·91 ± ·041	8	1·86 ± ·041
12	1·95 ± ·045	7	1·88 ± ·041
13	1·91 ± ·036	6	1·86 ± ·047
14	1·88 ± ·033	5	1·84 ± ·040
15	1·85 ± ·036	4	1·75 ± ·037
16	1·90 ± ·038	3	1·78 ± ·043
17	1·87 ± ·040	2	1·74 ± ·044
18	1·89 ± ·046	1	1·65 ± ·045

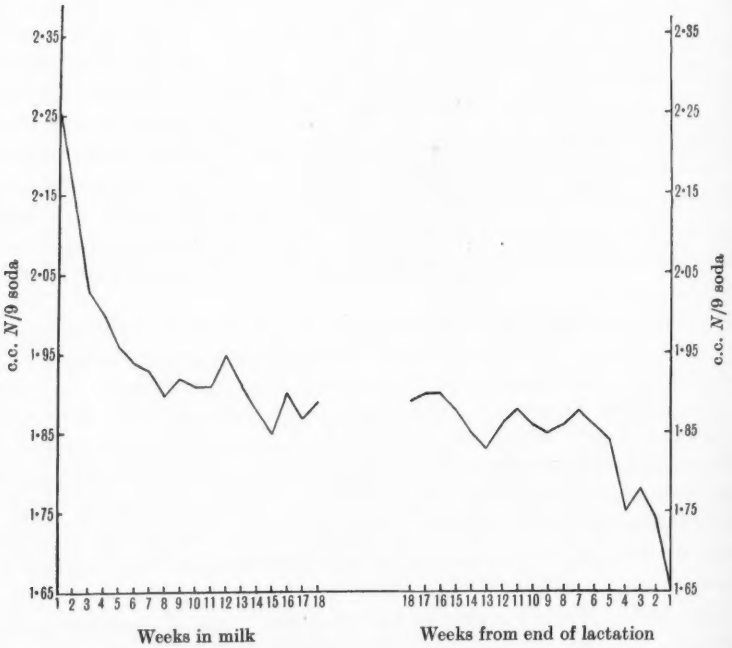


Fig. 1. The influence of the stage of lactation.

It will be seen from the graph that the acidity is highest at the beginning of the lactation and lowest at the end. The fall is not uniform but is fairly rapid during the first eight weeks of lactation and also during the last seven weeks. In the middle of the curve, from the eighth week of lactation until seven weeks before the milk flow ceases, the values are fairly uniform and lie between 1·95 c.c. $N/9$ and 1·85 c.c. $N/9$. The general shape of the curve is similar to that found by Mattick and Hallett (4).

The stage of lactation of the cow seems to be an important factor influencing the acidity of the milk of a cow. It is proposed to collect further data on acidity of milk and to make a statistical study of the factors which influence the average acidity of the milk of a herd.

SUMMARY.

Weekly tests of the acidity of the evening milk of each cow in the herd of the Agricultural Research Institute of Northern Ireland, Hillsborough, Co. Down, were made over a period of almost two years. A graph showing the influence of the stage of lactation on the acidity of the milk is given.

Thanks are due to Mr G. O'Donnell, of the Chemical Research Division, for the careful manner in which he carried out part of the experimental work.

REFERENCES.

(1) RICE, F. E. and MARKLEY, A. L. (1924). The relation of natural acidity in milk to composition and physical properties. *J. Dairy Sci.* **7**, 486.

(2) TOCHER, J. F. (1925). *Variations in the Composition of Milk*, p. 125.

(3) DUNCOMBE, E. (1924). The influence of certain factors on the hydrogen-ion concentration of milk. *J. Dairy Sci.* **7**, 86.

(4) MATTICK and HALLETT (1929). Studies in Lactation. I. *J. Dairy Res.* **1**, 35.

(*MS. received for publication*, 17. 2. 31.)

22. METHODS OF ESTIMATION OF SALT IN CHEESE

By F. H. McDOWALL and L. A. WHELAN.

Dairy Research Institute (N.Z.), Massey Agricultural College, Palmerston North, New Zealand.

Chlorine is present in cheese almost wholly as sodium chloride which in Cheddar cheese making is added at the last stage of the manufacturing process. Milk contains potassium chloride in somewhat larger proportion than sodium chloride, but only a very small quantity of these salts is carried over from the milk to the cheese. A cheese containing 36 per cent. of moisture would contain the chlorine present in 41 or 42 parts of the original milk. On the assumption that the chlorine content of the milk was 0·08 per cent. an unsalted cheese of the above moisture content should have a chlorine content of $41/100 \times 0{\cdot}08 = 0{\cdot}03$ per cent. (No allowance is made for salt added in the rennet.) For all practical purposes, therefore, the effect of differences in atomic weights of potassium and sodium on the chloride content as calculated from the chlorine content may be neglected. Estimation of salt in cheese is thus virtually a matter of estimation of the chlorine content.

The method to be used for the estimation of chlorine in organic materials depends to some extent on the nature of the material. Chlorine in pure organic compounds is usually estimated by either the Carius or the Stepanow method. Biological materials, on the other hand, generally contain sufficient mineral matter to fix the chlorine, and the most common method of chlorine determination has been the direct-ashing method. The method is not applicable where chlorine is present in organic combination, since a great deal of the chlorine may be lost during the incineration. In milk and other dairy products the amount of organically combined chlorine has not been determined, but the proportion is no doubt so small that it may be neglected.

Estimation by direct ashing.

Ashing of biological materials containing sodium chloride has to be performed with the utmost care if losses of chlorine through volatilisation of sodium chloride are to be avoided. In the estimation of ash in milk it is customary to extract the sodium and potassium chloride from the black char, before the final incineration to a white ash. Jensen and Plattner (1) consider that in the incineration of cheese some chlorine is displaced from combination with mineral matter by the phosphoric and sulphuric acids formed from the large proportion of casein present. Even extraction of the charred mass before the incineration to a white ash does not, in their opinion, completely obviate these losses, which they consider take place both during the burning and the incineration. They recommend preliminary treatment of the cheese with alkali:

"Mix the cheese intimately with a mixture of one part potassium nitrate and two parts of sodium carbonate, dry in a platinum crucible, and burn carefully." The chlorine in the residue is estimated volumetrically.

It is the experience of the authors of this paper that correct results *can* be obtained by the direct-ashing method without the addition of alkali, provided that the heating is carried out with the greatest care. It was not found possible, however, to obtain *consistently* accurate results by this method. The following procedure was finally adopted.

Method 1. Four grams of the prepared sample of cheese were weighed into a nickel crucible, and treated with 10 c.c. of sodium hydroxide solution (2*N*, from pure caustic soda, ex sodium; the use of stronger caustic soda was found to be inadvisable as it impeded the evaporation, owing to the formation of a gelatinous skin). The contents of the crucible were dried, first in the water bath and then in an electric oven at 110° C. The cheese was charred carefully over an Argand burner, small quantities (about 0·5 g. in all) of potassium nitrate being added from time to time to assist combination. No attempt was made to obtain a perfectly white ash. The residue was dissolved in 100 c.c. water, 15 or 20 c.c. of nitric acid (1 : 1) were added, and 20 c.c. *N*/10 silver nitrate. The mixture was then boiled to drive off nitrites, and the excess silver nitrate was titrated with *N*/10 thiocyanate solution. It was not found necessary to filter the silver chloride from the solution before titration, under the above conditions of titration (compare Whitehorn (2)).

The dry-ashing method is slow and tedious, and is not suitable for routine estimations. Cornish and Golding (3) estimated chlorine in cheese by a method which they claimed had advantages over the incineration method in that there was no necessity for long and careful heating, and no danger from loss of chloride. The cheese was heated in a Kjeldahl flask with concentrated sulphuric acid and the resulting hydrochloric acid gas was aspirated into a flask containing an acid solution of silver nitrate.

Estimation after precipitation of protein.

For the rapid estimation of chlorine in milk a number of methods have been devised. Apart from the electrometric method of Sundberg (*Zeit. f. Unt. der Lebensmittel*, 1928, **56**, 33), these methods may be divided into two classes.

(1) The chlorine is estimated by direct titration of excess silver nitrate, after precipitation of the protein with nitric acid.

(2) The protein is precipitated by nitric acid (Drost (4)), aluminium sulphate (Weiss (5)), or copper sulphate (Poetschke (6)), and the chlorine is estimated by titration in an aliquot portion of the filtrate.

The results recorded in the literature by the workers who have made comparisons of these methods do not show sufficient concordance to permit final estimates of their relative accuracy. It appears certain, however, from the work of van der Burg and Koppejan (7) that proteins must be removed by

filtration before the precipitation of chloride with silver nitrate. When silver nitrate is added to milk, some silver is carried down by the protein itself.

Van der Burg (8) recommends the following modification of these methods for estimation of salt in cheese:

Method 2. Four grams of the sample are weighed into a 100 c.c. measuring flask and 50 or 60 c.c. of water at 50–60° C., and 10 c.c. of normal caustic soda solution are added, the mixture being shaken until the cheese is dissolved. The solution is allowed to cool to room temperature, 10 c.c. of 6*N* nitric acid (sp. gr. 1·2) are added, the flask is filled to the mark with distilled water, and the mixture is filtered through a pressure filter. To 50 c.c. of the filtrate 15 c.c. of *N*/10 silver nitrate are added, and the excess silver is titrated back with *N*/10 thiocyanate solution in presence of 1 c.c. of saturated iron alum solution. The present authors have found it more convenient, for routine analysis, to heat the flasks in a boiling-water bath. This procedure hastens solution of the cheese by the caustic soda. It was also found more convenient to use fluted filter paper large enough to hold 100 c.c., in preference to a pressure filter, for separation of the precipitate from the solution. A rapid filtration was obtained, and the filtrate was clear.

Van der Burg calculates the salt content from the volume in c.c. of silver nitrate used, by means of the factor 0·2925, which is the theoretical factor on the assumption that the volume of liquid is 100 c.c. In 4 g. of cheese, however, there are about 2·5 c.c. of solid matter insoluble in the nitric acid solution. It would therefore be more correct to calculate the factor on the basis that the salt is dissolved in 97·5 c.c. of solution. The factor then becomes 0·285(2).

The method of van der Burg has been carefully tested at this Institute, and, in the opinion of the authors, gives very consistent results with the use of the factor 0·285.

Estimation after destruction of protein by nitric acid.

The principle of destroying protein by nitric acid in the presence of silver nitrate appears to have been first used by von Koranyi (9). He applied the method only to urine, and gave no indication of the degree of accuracy attained. Greenwald and Gross (10) have similarly deproteinised blood for chlorine determinations and have obtained results in good agreement with those obtained by other methods.

Van Slyke and Maclean (11), in their work on the estimation of chloride in blood, found that the precipitation of silver chloride in the protein-free picric acid filtrate was slow, and they suggested that the retardation might have been due to the presence of organic matter. They were successful in overcoming this disturbing influence by oxidation of the organic matter with nitric acid. Van Slyke (12) has described the following method for the estimation of chloride in blood and tissues, the complete operation being carried out in one vessel. The proteins are destroyed and the chloride precipitated, by heating with concentrated nitric acid in the presence of silver nitrate, the excess

silver nitrate being titrated by the Volhard method. Van Slyke uses 0·05*N* silver nitrate solution in concentrated nitric acid, and heating is continued on the water bath until the solution is clear and light yellow in colour. The time required for blood is about 12 hours. He finds it necessary to employ an empirical correction, which is subtracted from the reading for volume of thiocyanate, as a certain excess is needed to give a clear end-point.

An adaptation of the above method of Van Slyke to the estimation of salt in cheese has been found by the present authors to give results in good agreement with those found by the ashing method, the complete estimation requiring only about 15 minutes.

Method 3. Four grams of the cheese sample are weighed into an Erlenmeyer or Kjeldahl flask, 20 c.c. of *N*/10 silver nitrate and 10 c.c. of concentrated nitric acid are added. The mixture is boiled over a free flame in a fume cupboard for 5 minutes, diluted with distilled water (50 c.c.), and cooled. One gram of powdered iron alum is added, and the excess silver nitrate is titrated with *N*/10 thiocyanate. Some difficulty may at first be experienced with this titration, on account of the pronounced yellow colour of the solution due to the presence of nitro-compounds. With practice, however, the end-point can be detected quite accurately in daylight. It is advantageous to compare the solution being titrated with a similar solution to which no thiocyanate has been added.

EXPERIMENTAL RESULTS.

The three methods outlined above were tested in two series of experiments. In the first series known amounts of salt were added to weighed quantities (4 g.) of a homogeneous sample of an unsalted cheese. In the second series the salt was estimated in a homogeneous sample of a salted cheese, and in further portions of the same sample, to each of which known quantities of salt had been added. The difference between the salt contents of the treated and untreated cheese, as found by each method, was compared with the amount of salt added. Salt was added in the form of a solution (0·137 *M*) prepared from recrystallised Merck sodium chloride *puriss.*, dried in an air oven at 110° C. Duplicate or triplicate estimations were carried out in every case. The results are tabulated below.

Method No. 1.

Unsalted cheese		Salted cheese	
NaCl added (g.)	NaCl estimated (g.)	NaCl added (g.)	NaCl estimated (g.)
0	0·002	0	0·068
0	0·002	0	0·065
0	0·004	0·040	0·108
0·040	0·042	0·040	0·109
0·040	0·043		
0·040	0·043		
0·040	0·042		

Method No. 2.

Unsalted cheese		Salted cheese	
NaCl added (g.)	NaCl estimated (g.)	NaCl added (g.)	NaCl estimated (g.)
0	0·002	0	0·073
0	0·002	0	0·070
0·040	0·043	0·040	0·111
0·040	0·041	0·040	0·110
0·080	0·081	0·040	0·112
0·081	0·082	0·040	0·112
0·120	0·121		
0·120	0·121		
0·160	0·161		
0·160	0·162		

Method No. 3.

Unsalted cheese		Salted cheese	
NaCl added (g.)	NaCl estimated (g.)	NaCl added (g.)	NaCl estimated (g.)
0	0·002	0	0·071
0	0·001	0	0·073
0·040	0·042	0·040	0·113
0·040	0·041	0·080	0·153
0·080	0·083	0·120	0·194
0·080	0·083	0·120	0·193
0·080	0·084		

The following determinations were made at the same time by the three methods on the same homogeneous sample of salted cheese.

NaCl added (g.)	NaCl estimated (g.)		
	Method 1	Method 2	Method 3
0	0·075	0·078	0·077
0	0·077	0·078	0·076
0	—	0·078	0·076
0·048	0·124	0·124	0·124
0·048	0·125	0·125	0·123
0·048	—	0·127	0·124

SUMMARY.

Consideration has been given to the various methods available for the estimation of salt in cheese. The following three methods have been found to give concordant results, and to permit accurate estimation of known added quantities of chloride.

(1) Careful ashing with small amounts of potassium nitrate, after addition of some dilute caustic soda, and subsequent titration of chloride according to the method of Volhard.

(2) Solution of the cheese in caustic soda, precipitation of the proteins by nitric acid, and titration (according to Volhard) of chloride in an aliquot portion of the filtrate (van der Burg's method, with some modifications introduced by the authors).

(3) Titration by the Volhard method after destruction of the proteins by hot nitric acid in the presence of excess silver nitrate.

Methods (2) and (3) have been found suitable for routine analysis of cheese.

REFERENCES.

(1) Jensen and Plattner (1906). *Zeit. Unt. Leb.* **12**, 195.
(2) Whitehorn (1920). *J. Biol. Chem.* **45**, 449.
(3) Cornish and Golding (1915). *Analyst*, **40**, 197.
(4) Drost (1923). *Zeit. Unt. Leb.* **45**, 247.
(5) Weiss (1921). *Mitteil. aus dem Gebiete der Lebensmitteluntersuchungen u. Hygiene*, **12**, 133 (after Drost, *loc. cit.*, 252).
(6) Poetschke (1910). *Ind. Eng. Chem.* **2**, 210.
(7) van der Burg and Koppejan (1924). *Le Lait*, **4**, 32, 81.
(8) van der Burg, *Tijdschr. u. vergelijkende Geneesk.* **15**.
(9) von Koranyi (1897). *Z. klin. Med.* **33**, 1.
(10) Greenwald and Gross (1922). *J. Biol. Chem.* **54**, 589.
(11) Van Slyke and Maclean (1923). *J. Biol. Chem.* **56**, 813.
(12) Van Slyke (1923). *J. Biol. Chem.* **58**, 523.

23. RUSTY SPOT IN CHEDDAR AND OTHER CHEESE. III

A. FURTHER FACTORS CONTROLLING PIGMENTATION

By A. T. R. MATTICK, B.Sc., Ph.D. and J. G. DAVIS, M.Sc.

B. THE APPLICATION OF THE RESULTS OF LABORATORY INVESTIGATIONS TO CHEESE-MAKING

By A. T. R. MATTICK, B.Sc., Ph.D., J. G. DAVIS, M.Sc., and D. V. DEARDEN, N.D.D., B.D.F.D.

National Institute for Research in Dairying, University of Reading

(With 1 Figure.)

In two previous papers the authors(1, 2) have dealt with the nature of the organism causing "Red" or "Rusty" Spot in hard-pressed cheese from various parts of the Empire and have described some of the conditions controlling the formation of pigment. The organism has been shown to have the closest affinities with the true lactic acid bacteria, only differing from them in its ability to produce a red-brown pigment under much reduced oxygen tensions and in the presence of substances containing vitamin B_1 with which, however, the pigment-producing factor is not identical(3). In addition, the presence of a fermentable carbohydrate and a minimal concentration of peptone nitrogen have been shown to be necessary, whilst most abundant pigmentation occurs at an interface and in the presence of free moisture. It has also been shown that some peptones are quite inadequate for pigment formation.

The present paper deals in the first part with further work upon the conditions governing pigment formation, and in the second with its application to cheese-making.

A. Further factors controlling pigmentation.

Repeated attempts to secure rapid pigmented growths of the organism in the ordinary laboratory media at various *p*H values and oxygen tensions have failed. For example, in sterile separated milk growth is very slow, at least a month being required to produce appreciable pigment, the intensity of which is, however, ultimately excellent. Since a bright pigment often occurred in cheese from infected farms it appeared that some essential condition or substance must be absent from sterile separated milk, and attempts were made to repair the deficiency. In the first instance rennet was added to the sterile separated milk and a well-pigmented growth obtained in about 24 hours. It was possible that this rapid growth was due to the scission and degradation of the casein, but the rennet contained a variety of micro-organisms which might have played a part in the reaction. That something more than the addition of rennet was necessary for pigment production had

been demonstrated under practical cheese-making conditions in which cheese made from milk coagulated by rennet and heavily inoculated with cultures of the causative organism, either failed entirely to produce the fault or contained only an insignificant number of minute spots.

Moreover, tryptic and peptic digests of chemically purified casein did not permit pigmented growth, whereas a peptic digest of ordinary rennet-precipitated curd from naturally contaminated milk gave good growth and pigment. This behaviour of tryptic and peptic digests is in accord with the findings of other workers (4, 5) who showed that purification of natural proteins resulted in the removal of factors responsible for the stimulation of growth.

It therefore appeared that rennet, without its accompanying organisms, was unable to stimulate pigment formation. However, to confirm this, rennet was filtered through a sterile candle, and the filtrate added to separated milk. This medium was inoculated with the organism. The results of the experiment are shown in Table I.

Table I.

Sterile milk + red spot organism	No pigmented growth
Sterile milk + rennet filtrate + red spot organism	„
Sterile milk + rennet filtrate	Coagulation
Agar slope + rennet filtrate	No growth
Sterile milk + unfiltered rennet + red spot organism ...	Rapid pigmented growth

It may therefore be concluded from these results that the phenomena observed when raw milk was used as a medium were due to the accompanying organisms.

In order to follow up this conclusion samples of raw milk, drawn under aseptic conditions into test-tubes, were inoculated with the red spot organism and incubated at 22° to 30° C. Within 16 hours an abundant and well-pigmented growth of the organism had appeared. This experiment was repeated many times with the same result. It was, however, subsequently found that not all raw milk would give rapid pigmentation.

On boiling the milk for 20 minutes, cooling and then inoculating with the red spot organism, it was found that no pigmented growth took place even after protracted incubation. A variety of experiments such as these was carried out with milk from different sources. The results are shown in Table II.

Table II. *Growth of red spot organism in raw milk.*

Raw milk from a number of individual cows	Growth and bright pigment after 16 hours at 22° C.
The same raw milk boiled	No apparent growth; no pigment[1]
Colostrum (raw)	Growth and bright pigment after 15 hours at 22° C.
„ (boiled)	No apparent growth; no pigment[1]
Mastitis milk (raw)[2]	Excellent growth and pigment in 24 hours at 22° C.
„ (boiled)	No apparent growth; no pigment[1]
Controls (uninoculated raw milk)	Peptonisation after clotting; no pigment in any case

[1] No pigment visible after 2 months.
[2] The pigment formed in mastitis milk was consistently better than that in normal raw milk.

It might be argued from the results in Table II that the pigment-promoting factor was present in raw milk at the time of secretion but was destroyed on boiling. That this is probably not so is shown by the result of various cheese-making experiments, in which large volumes of raw milk were inoculated with emulsions of the red spot organism. The ripe cheese were non-pigmented. In order, however, to confirm this conclusion a bacteria-free filtrate of fresh raw milk was used as a medium. No growth or pigmentation took place. It may be concluded, therefore, that the marked growth and pigmentation recorded in Table II were due to the presence of the growing bacteria in the unheated milk. The growth and pigmentation were much more rapid than those obtained by the addition of yeast extract to sterile separated milk (2).

To verify this assumption, the organisms present in the raw milk first used, and in that from other sources, were isolated by plating on various media and tested for their pigment-stimulating properties. Table III gives some of the reactions and characteristics of these organisms.

It was found that about half of the organisms when added to a culture of the red spot organism in sterilised milk gave a pigmented growth in 24 hours, as in the case of the raw milk. This stimulation may be due (*a*) to the degradation of protein matter to more readily assimilable forms, (*b*) to the

Table III. *Characteristics and reactions of stimulating and non-stimulating organisms.*

Organism	Morphology	Gram reaction	Type of growth	Catalase	Action on litmus milk	Stimulation of pigmentation
B. subtilis	Sporing bacillus	+	Surface	+	Alk., peptonised	–
Paratyphoid B	Short rod	–	Surface	+	Acid, subseq. alk.	–
B. dysenteriae	Short rod	–	Surface	+	—	+
Proteus vulgaris	Short rod	–	Surface	+	Peptonised	–
Bact. aerogenes	Short rod	–	Surface	+	Acid, clot	+
Coliform	Short rod	–	Surface	+	Acid, clot	+
Propioni bacterium	Plump rod	+	Depth	+	Acid, clot	–
D 1	Short rod	–	Surface	+	Acid, clot	–
D 2	Large rod	–	Surface	+	Acid	–
D 3	Long rod	+	Surface	+ +	Alk., peptonised	+
D 4	Short rod	–	Surface	+	Acid	–
D 5	Short rod	–	Surface	+	Alk., peptonised	+
D 9	Short rod	–	Depth	–	Alk., peptonised	+
D 10	Short rod	–	Depth	+	Alk., peptonised	+
D 12	Short rod	–	Depth	+	Alk., peptonised	+
G 1	Yellow micrococcus	+	Surface	+	—	–
G 2	Orange coccus	+	Surface	+	Acid, clot	–
G 3	Coccus	+	Surface	+	Alk.	–
G 4	Short rod	–	Surface	+	Acid, clot, peptonised	–
G 5	Short rod	–	Surface	+	—	–
G 6	Short rod	–	Surface	+	Acid	+
G 7	Short rod	+	Surface	tr.	Acid, clot, peptonised	–
G 8	Diphtheroid	+	Surface	+	Alk.	–
G 9	Rod	–	Surface	+ +	Acid, clot, peptonised	+

Summary of results of 27 additional organisms isolated from milk of the Institute's herd.

Type	No used	Gram reaction	No stimulation	Stimulation
Tetracocci	2	+	2	0
Staphylococci	3	+	0	3
Micrococci	2	−	0	2
Coccobacteria	4	−	1	3
Short bacteria	4	+	4	0
Diphtheroids	2	+	1	1
Sporing bacilli	5	+	2	3
Yeasts	2	+	0	2
Rods	2	−	0	2
Diplococcus	1	+	0	1

production of bacterial "growth factors" or (*c*) to physical conditions induced by the growth of the organisms.

With the evidence at present available the first explanation must be left an open question, although in the light of other observations and especially of the properties of the stimulating and non-stimulating types of bacteria isolated, it appears unlikely that protein degradation is the sole cause of the stimulation observed (10).

The theory that the stimulation is due to the production of "growth factors" is supported by the knowledge that many different types of bacteria are able to produce vitamin B, with which our factor is known to be associated (2, 3). A specific instance is afforded by the fact that *Flavobacterium vita-rumen*, which has been shown to produce vitamin B in the rumen of a cow (6), induced rapid pigmentation of the red spot organism in sterilised milk. It was found, however, that a heated and sterilised culture of a stimulating organism in the basal medium (2) did not permit pigmented growth. It may be concluded therefore that the stimulation is a function of the living organism.

The third possibility was investigated by studying the oxidation-reduction potential induced in sterile milk by the growth of stimulating and non-stimulating organisms isolated from milk from various sources. It was found that those organisms which stimulated growth and pigmentation always induced a highly negative potential, whereas those which did not stimulate growth and pigmentation attained potentials considerably more positive (Fig. 1). It should be borne in mind that the oxidation-reduction potential induced by living cultures of bacteria is a function, not only of the metabolic uptake of oxygen, but of reducing substances resulting from the metabolism. It is possible, as suggested in a previous paper (2), that the second and third hypotheses are not unrelated. It may be recalled that those tissues which had previously been found to stimulate pigmentation and to contain vitamin B, were also rich in those substances, such as glutathione, which exert a marked effect on the oxidation-reduction potential.

It is not claimed that the ability to induce specific oxidation-reduction conditions is the only factor involved in pigment formation, but it is the only

one which can be correlated with pigment stimulation in all the organisms examined.

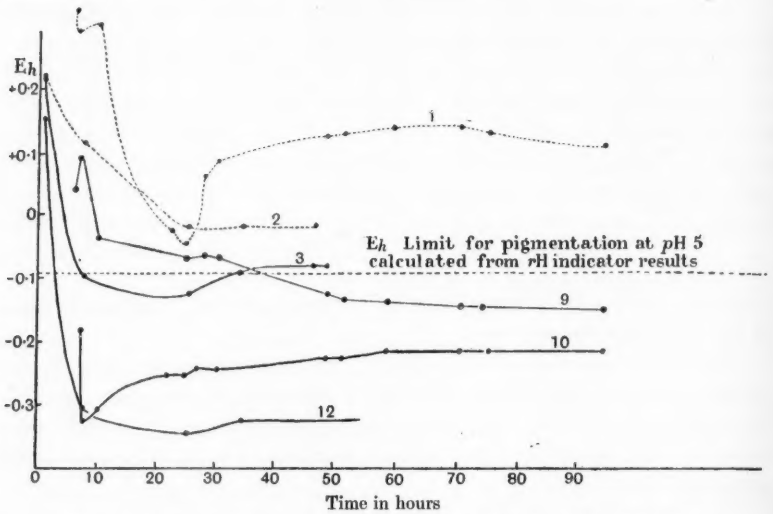


Fig. 1. Time-potential curves of organisms 1, 2, 3, 9, 10, and 12. (Table III.)
- - - - = no pigment stimulation when grown with red spot organism.
—— = pigment stimulation when grown with red spot organism.

B. The application of the results of laboratory investigations to cheese-making.

Although red spot has been observed in nearly all varieties of English hard-pressed cheese it has been found to be most extensive in those which are moist and open in texture. This is particularly true of Cheddar cheese in which the size and number of red spots are at a minimum when the texture is close and the cheese are true specimens of their type.

In view of these facts a series of cheese-making experiments was carried out in which the method of manufacture was deliberately varied in order to obtain cheese of widely differing physical characters. It was hoped to be able to correlate the extent of red spot infection with the physical characters of the cheese made from milk inoculated with the causative organism.

With this object in view normal, sweet, acid, wet, overscalded and underscalded cheese were made. In the majority of cases the milk used came from the mixed herd of Shorthorns and Guernsey cows at this Institute, and was subject to strict bacteriological control and contained very small numbers of micro-organisms except when otherwise stated (Table IV). The significance of the bacteriological count only became apparent as the laboratory work upon the red spot organism progressed and was not appreciated when the earlier experimental cheese were made. In the light of later knowledge it must be considered fortunate that the milk used came from a controlled source.

First series of experimental cheese.

The results of the first series of experiments are shown in Table IV.

The milk was always inoculated with large numbers of the red spot organism which had been grown in the special media devised, but variations were made in the method of cheese manufacture. Uninoculated control cheese were made with milk from the same source on each occasion. The cheese were ripened in a good ordinary cheese cellar and examined at intervals by taking borings. Before disposal, all experimental cheese were cut through and thoroughly examined for red spot.

Table IV. *Cheese made from inoculated milk from controlled sources. Variations in the method of manufacture.*

Date	Method of manufacture	Source of milk	Results of examination for red spot
1925			
Nov. 13	Normal	Institute farm	None
Nov. 20	Acid	Institute farm	None
Dec. 1	Sweet	Institute farm	None
1926			
*March 2	Normal	Institute farm	Very numerous red spots
May 7	Sweet and wet	University farm	A very few suspicious areas not definite red spot
May 10	Sweet and wet	University farm	A very few suspicious areas not definite red spot
July 2	Normal	Institute farm	None
1927			
Jan. 21	Normal	Institute farm	None
Jan. 24	Normal	Institute farm	Numerous red spots
Jan. 26	Sweet and wet (No starter)	Institute farm	None
Jan. 28	Sweet and wet (No starter)	Institute farm	None
Jan. 31	Sweet	Institute farm	None
1928			
March 7	Normal	Institute farm	None
March 7	Acid	Institute farm	None
March 7	Sweet	Institute farm	None
Apr. 10	Normal	Institute farm	One or two tiny spots
Apr. 10	Sweet	Institute farm	One or two tiny spots
Apr. 10	Double quantity starter	Institute farm	A few small spots
Apr. 10	Overscalded	Institute farm	A few small spots
Apr. 10	Underscalded	Institute farm	One or two tiny spots
May 25	Normal	Institute farm	Numerous red spots
May 25	Sweet	Institute farm	Numerous red spots
May 25	Acid	Institute farm	One or two tiny spots

* Milk taken by machine during experiments on methods of cleansing contained large numbers of bacteria (see ref. 7).

It is clear from Table IV that with few exceptions the amount of red spot found in the cheese, in spite of heavy inoculation with the causative organism, was nil or negligible from the practical standpoint. To meet the possibility that the cultures used were not viable it should be stated that on every occasion when an examination was made the red spot organism could be recovered from the press-whey or the ripe cheese (which were themselves free from red spot), and could be made to form pigment on appropriate media.

The cheese of March 2nd, 1926 (Table IV), is significant. The milk used was

known to be heavily contaminated (7), and resulted in the appearance of numerous red spots. This result is in accord with that obtained in the laboratory and reported in the first part of this paper.

It appears, therefore, that no amount of manipulation of the method of manufacture can be made to give typical red spot cheese consistently, and the explanation of its occurrence in practice had to be sought elsewhere.

Second series of experimental cheese.

The failure to secure typical red spot cheese by varying the method of manufacture when using the milk of the Institute's herd led to a repetition of the experiments with milk from other sources, since it was possible that necessary factors might have been absent from the milk of one farm. Variations in the method of manufacture were continued in this series, the results of which are reported in Table V.

Table V. *Cheese made from inoculated milk from various sources. Variations in the method of manufacture.*

Date	Method of manufacture	Source of milk	Results of examination for red spot
1928			
July 14	Normal	British Dairy Institute	None
*July 26	Normal	Farmers' Clean Milk Dairies (Grade A (T.T.) milk)	None
Aug. 27	Normal	British Dairy Institute, I	None
Aug. 27	Normal	British Dairy Institute, II	None
Aug. 31	Normal (not inoculated)	Red Spot farm I	A few small spots
Aug. 31	Sweet (not inoculated)	Red Spot farm I	A few small spots
Aug. 31	Acid (not inoculated)	Red Spot farm I	A few tiny spots
Oct. 3	Wet (not inoculated)	Red Spot farm I	A few tiny spots
Oct. 3	Normal (not inoculated)	Red Spot farm I	A few tiny spots
Oct. 4	Wet (not inoculated)	Red Spot farm I	A few tiny spots
Oct. 4	Normal (not inoculated)	Red Spot farm I	A few tiny spots
*Oct. 29	Normal	Farmers' Clean Milk Dairies (Grade A (T.T.) milk)	None
Oct. 29	Normal	Farmers' Clean Milk Dairies (Grade A (T.T.) milk)	A very occasional tiny spot
*Nov. 7	Gruyère cheese method (not inoculated)	Farmers' Clean Milk Dairies (Grade A (T.T.) milk)	None
*Nov. 7	Gruyère cheese method	Farmers' Clean Milk Dairies (Grade A (T.T.) milk)	One or two tiny spots
*Nov. 12	Gruyère cheese method (not inoculated)	Farmers' Clean Milk Dairies (Grade A (T.T.) milk)	None
*Nov. 12	Gruyère cheese method	Farmers' Clean Milk Dairies (Grade A (T.T.) milk)	One or two tiny spots
*Dec. 5	Normal	Farmers' Clean Milk Dairies (Grade A (T.T.) milk)	None

* Milk subject to bacteriological control.

It will be seen, as in the previous experiment, that the amount of red spot found in the cheese made by various methods is nil or negligible in spite of the fact that the milk came from a variety of sources. It appeared, therefore, that no variation of the physical condition of the curd could be relied upon to give typical red spot infection in cheese made from inoculated milk. In no case was a cheese made in which an amount of red spot even remotely approximating to that found in ordinary farm practice developed.

Third series of experimental cheese.

The laboratory experiments had shown that certain substances induced pigment formation when added to a non-pigmenting medium. A third series of cheese-making experiments was therefore undertaken in which the cheese were made by the wet method and various substances known to affect pigment formation added at different stages of the cheese-making process.

The results of these experiments are shown in Table VI.

Table VI.

Date	Method of manufacture	Source of milk	Results of examination for red spot	Substance added during manufacture
1928				
Dec. 5	Normal	Institute farm	None	*Carotin*. Culture added
Dec. 5	Normal	Institute farm	None	Culture added
1930				
March 31	Wet	Institute farm	A few small spots	*Saccharose* and culture added to the cut curd
March 31	Wet	Institute farm	A few small spots	Culture of red spot organism added to the cut curd
March 31	Wet	Institute farm	Numerous red spots	Culture of red spot organism in raw milk added to the cut curd
March 31	Wet	Institute farm	Numerous red spots	*Saccharose* and a culture of red spot organism in raw milk added to the cut curd
Apr. 2	Wet	Institute farm	Very numerous, evident spots	Culture in raw milk added to cut curd
Apr. 2	Wet	Institute farm	Very numerous, evident spots	50 % *extra salt* and culture in raw milk added to the cut curd
Apr. 2	Wet	Institute farm	Very heavy infection with red spots, cheese unsaleable	Culture in raw milk added with normal quantity of salt to cut curd
Apr. 2	Wet	Institute farm	Numerous red spots	50 % *extra salt* and culture in raw milk added to the cut curd
Apr. 9	Wet	Institute farm	A few small spots	Culture in sterile milk added to the milk
Apr. 9	Wet	Institute farm	None	50 % *extra salt* and culture in sterile milk added to milk
Apr. 9	Wet	Institute farm	Very numerous, evident spots	Culture in raw milk added to milk
Apr. 9	Wet	Institute farm	None	50 % *extra salt* and culture in raw milk added to milk
Apr. 3	Wet	Institute farm	Numerous red spots	Sterile milk culture added
Apr. 3	Wet	Institute farm	Numerous red spots	Sterile milk culture and *yeast extract* added
Apr. 3	Wet	Institute farm	Numerous red spots	*Raw* milk culture added
Apr. 3	Wet	Institute farm	Numerous red spots	Raw milk culture and *yeast extract* added
Apr. 4	Wet	Institute farm	One or two tiny spots	Sterile milk culture added
Apr. 4	Wet	Institute farm	Some small spots	Sterile milk culture and *yeast extract* added
Apr. 4	Wet	Institute farm	Some small spots	Raw milk culture added
Apr. 4	Wet	Institute farm	Numerous red spots	Raw milk culture and *yeast extract* added

(*a*) *Carotin.*

The fact that the pigment of the red spot organism appears to be carotinoid in nature suggested that carotin and its associated pigments, if added to the milk, might increase the amount of pigment formed by the organism. No evidence of this possibility was obtained.

(*b*) *Saccharose.*

The addition of this sugar to the curd has been stated (8) to inhibit a reddish brown discoloration caused by certain organisms, which, however, were evidently fundamentally different from the organism now described. Saccharose was found to have no inhibiting effect on the red spot organism.

(*c*) *Salt* (NaCl).

Laboratory experiments had shown that pigmentation could be decreased and even in some cases inhibited, by increasing the salt concentration in the pigment-producing medium. The amount of salt added was, as shown in Table VI, increased by 50 per cent. and definite inhibition of red spot formation was produced.

In order to test the possibility of applying these results to practice a number of experiments was carried out in which the salt concentration was varied. The results are given in Table VII and show that when the quantity of salt is increased by 50 per cent. of the normal, some decrease in the amount of red spot is found. When the amount of salt is double the normal quantity the incidence of red spot is halved, but the cheese are unsaleable. This method of controlling the fault is therefore impracticable.

Table VII.

Quantity of salt	Number of cheese	Comparative incidence of red spot
1 oz. to 3 lb. curd	10	2·5
1 oz. to 2½ lb. „	10	2·5
1 oz. to 2 lb. „	10	2·0
1 oz. to 1½ lb. „	10	1·3

(*d*) *Yeast extract.*

In laboratory experiments yeast extract was shown (2) to favour the growth of the red spot organism. Some increase in the amount of red spot in the inoculated cheese was in fact found in the experiment of April 4th, 1930 (Table VI), but its extent was so small as to make it probable that the substances added with the yeast extract were not the only ones necessary.

(*e*) *Raw milk cultures of the red spot organism.*

If the incidence of red spot in the cheese described in Table VI be compared with that in Tables IV and V the differences will be apparent.

In many of the experiments in the third series a raw milk culture of the red spot organism was used for inoculation, since great acceleration of pigment

formation had been shown to occur when other organisms were allowed to grow with the red spot organisms in milk. The much greater amount of the red spot which this method induced, compared with that obtained when sterile milk cultures were used, is made evident when the experiments reported in Tables IV and V and those in Table VI are compared.

Influence of hydrogen-ion concentration on the incidence of the fault.

In investigating the possibility that the degree of acidity developed in the cheese might have an effect upon the pigmentation of the red spot organism a number of determinations of the hydrogen-ion concentration of the ripe cheese was made by means of the quinhydrone electrode. In Table VIII the average incidence of red spot in a number of groups of cheese is set out, with the corresponding average *p*H. All the cheese so examined were made from the milk of the same herd.

The differences between badly infected and non-infected cheese are so small as to be without significance except to confirm previously reported results (1) in which the limit was shown to be as low as *p*H 3·5 to 4. Unlike coliform and similar organisms, therefore, the *p*H of normal Cheddar cheese is well within the range of growth of this organism.

It is also of interest to note that these results agree closely with those reported for Cheddar cheese by Lundstet (9).

Table VIII. *Hydrogen-ion concentrations of ripe cheese.*

Comparative incidence of red spot	Number of cheese	Average *p*H
–	7	5·24
+	8	5·22
+ +	9	5·27
+ + +	5	5·17
+ + + +	1	5·27

Discussion.

The results of the cheese-making experiments reported in Tables IV, V and VI seem to warrant certain conclusions.

(1) The non-appearance of red spot in cheese made from the milk of the Institute's farm was not due to any special deficiency in the milk, since mixed milk from several other sources also failed to promote pigment formation.

(2) The method of manufacture and the resulting texture of the cheese are not the most important factors in the production of red spot, although they have some effect.

(3) Pigment could be neither greatly diminished nor increased by adding at various stages in the manufacture of the cheese a variety of substances previously shown to influence pigmentation.

(4) Inoculation with a raw milk culture of the causative organism produced much more red spot than was obtained with a culture in sterile milk.

The failure to secure typical red spot cheese with the milk of the Institute's farm and that from other farms, even when the milk was heavily inoculated with the causative organism, led to the conclusion that some factor which was present during cheese-making at an infected farm was absent when the cheese were made in the Institute's dairy.

Further, Table V shows that milk from an infected farm, when taken under strictly controlled conditions, even though it contained the red spot organism, showed an insignificant amount of infection when cheese was made from it at the Institute's dairy.

It has, however, also been shown that if the culture of the red spot organism used for inoculation contained other organisms found in raw milk, the resulting cheese was heavily infected. This, coupled with the laboratory experiments, suggested that the miscellaneous flora of the milk played an important part in determining the extent of red spot infection.

With these facts in mind the experiment of October 7th, 1930 (Table IX), was carried out.

Table IX. *Milk taken under various conditions on a "red spot farm."*

Date	Method of manufacture	Source of milk	Results of examination for red spot	Substances added
Oct. 7	Normal	Red Spot farm II	None	No culture added. Milked in the fields with special precautions (see text) and cheese made at the Institute's dairy
Oct. 7	Normal (farm method)	Red Spot farm II	Very numerous evident red spots	No culture added. Milk taken in the ordinary way and cheese made at the farm
Oct. 8	Normal (farm method)	Red Spot farm II	Very heavy infection with red spots	No culture added. Milk taken in the ordinary way and cheese made at the farm

The herd at an infected farm was divided into two parts, one of which was milked in the fields into sterile utensils with all the precautions usually observed to secure clean milk, and the other half of the herd was milked in the sheds in the manner usual at the farm.

The milk of the first half was sent to this Institute and made into cheese and the other dealt with at the farm by the usual cheese-making methods. It was found that no red spot appeared in the cheese made at this Institute, but that the cheese made at the farm was heavily infected. Further, the cheese of the next day made in the usual way at the farm was also badly infected. Some of the milk used for the cheese made at this Institute was kept and shown to contain the red spot organism, although no trace of the fault appeared in the cheese.

It is probable that the severity of the infection of the cheese made at the infected farm was partly due to the presence in the utensils of numbers of the red spot organism. The only other significant difference between this and the

cheese made at the Institute was the care taken to exclude contamination during milking in the case of the latter.

It would be unwarrantable to draw conclusions from a single experiment, but the importance of the miscellaneous flora of milk becomes evident when it is considered that on all the occasions during experimental cheese-making when red spot was appreciable, the bacterial count of the milk was high owing to chance or deliberate contamination (Tables IV, V, VI).

The tentative conclusion may therefore be drawn, that if on a farm infected with the red spot organism the cheese milk contains numerous miscellaneous organisms which are allowed to grow (as, for example, in farm practice where the night's milk is uncooled and is kept in a warm dairy), the necessary conditions for pigmentation by the red spot organism will be created and infected cheese result.

The remarkably rapid pigmentation of the organism on every occasion when added to mastitis milk suggests that if any appreciable proportion of the cheese milk comes from diseased udders the chances of the cheese being infected with red spot will be greatly increased.

The subsequent addition of starter cannot, of course, counteract the results of the growth of other organisms, but, because it reduces the oxidation-reduction potential, may even encourage it.

The ultimate habitat of the organism remains as yet undiscovered in spite of an intensive search in a great variety of possible situations.

Meanwhile the work so far recorded suggests that the incidence of red spot may be controlled by excluding bacteria from milk during milking and rejecting milk from diseased udders.

Investigations of the modes of infection of the dairy and the production of cheese free from the fault on a red spot farm will be described in a later paper.

The authors wish to acknowledge the help received in the making of the cheese, from Miss E. M. Hallum, Miss M. Sellers and Miss F. Chapman, and to thank Dr S. K. Kon for a culture of *Flavobacterium vita-rumen*.

Summary.

1. The most important factor in the growth of the red spot organism in milk is the presence and growth of certain other bacteria. The reason for this is discussed.

2. The bacteria most active in metabiotic stimulation of pigment production are those inducing low oxidation-reduction potentials in milk.

3. The influence of factors such as variations in the method of manufacture and the composition of the milk and curd on the incidence of the fault have been investigated under practical cheese-making conditions.

4. There is no correlation between the hydrogen-ion concentration and other properties of the ripe cheese and the extent of the red spot.

5. Measures for the control of red spot have been suggested.

REFERENCES.

(1) DAVIS and MATTICK (1929). *J. Dairy Res.* **1**, 50.
(2) —— —— (1930). *Ibid.* **1**, 136.
(3) DAVIS and GOLDING (1930). *Biochem. J.* **24**, 1503.
(4) FREEDMAN and FUNK (1922). *Proc. Soc. Exp. Biol. and Med.* **19**, 198.
(5) MUELLER (1922). *J. Bact.* **7**, 309.
(6) BECHDEL et al. (1928). *J. Biol. Chem.* **80**, 231.
(7) MATTICK and PROCTER (1928). *J. Hygiene*, **27**, 215.
(8) Leeds University (1909). *Bull.* **77**.
(9) LUNDSTET (1930). *Maelkeritidende*, **43**, 45, 937.
(10) DAVIS and MATTICK (1930). *Zbl. f. Bakt.* Abt. II, **80**, 30.

24. A STUDY OF *S. PARACITROVORUS* AS A STARTER IN BUTTER-MAKING

By E. C. G. MADDOCK, C.I.E., M.D.

National Institute for Research in Dairying, University of Reading.

Owing to the deteriorations produced (Rogers(1)) in the keeping quality of butter churned from ripened cream, the practice of using starters has been abandoned to a great extent in some large butter-making areas of the world. This has resulted in a product which is undoubtedly lacking in that flavour which is so highly appreciated by the consumer. It is not now disputed that much of the flavour in butter arises from the formation of traces of volatile acids, their esters and other by-products during fermentations induced by *S. lactis* or *S. cremoris* and those organisms, such as *S. citrovorus* and *S. paracitrovorus* (Hammer(2)), commonly associated with them in starters.

The latter organisms are distinguished from *S. lactis* chiefly by their production of considerable volatile acidity, their action upon citrates and the comparatively low total acidity ordinarily produced in milk.

It appears that neither *S. citrovorus* nor *S. paracitrovorus* can, when used as pure culture starters, be expected to yield butter of better flavour than when combined with *S. lactis* in a good starter (Hammer(2)). However, as suggested by Hammer, it was considered possible that the very fact that the acid-producing powers of *S. citrovorus* and *S. paracitrovorus* are low, might make it possible to produce a fine-flavoured butter which would not be subject to such rapid deterioration as that made from cream ripened by *S. lactis* and *S. cremoris*. The experiments described in this paper were, therefore, carried out to determine the practical value of pure cultures of *S. citrovorus* and *S. paracitrovorus* as starters. Very early in the experiments it became obvious that *S. citrovorus* alone would not make a practicable starter because of its low acid-producing powers and inability to compete with the inevitable contaminants encountered in practice. The work was, therefore, latterly confined to the use of *S. paracitrovorus*.

The strains of this organism which were used in the first instance were kindly supplied by Dr B. W. Hammer, Iowa State College of Agriculture, U.S.A. Later, strains isolated locally, which gave all the reactions (Hammer(2)) of *S. paracitrovorus*, were used.

Preparation of *S. paracitrovorus* starter.

Because of the comparatively low acid-producing powers of pure cultures isolated from starter or dung it was found necessary to make repeated sub-cultures into separated milk. By this means the original power of acid

production was increased sufficiently to warrant the use of such a culture for starter preparation.

Using six strains (A–F) which had been repeatedly grown in sterile milk, inoculations were made into 150 c.c. quantities of fresh pasteurised whole milk which were then incubated at 22° C.

The acidities recorded after 24 hours are shown below.

Table I. *Percentage acid, calculated as lactic acid.*

Strain	After 24 hours	After 48 hours
A	0·730	0·920
B	0·720	0·860
C	0·780	0·860
D	0·780	0·940
E	0·820	0·920
F	0·260	0·900

It will be seen that some of the acidities produced are in the region of those induced by *S. lactis* and, although not exceptional for *S. paracitrovorus*, are higher than many of those reported by (Hammer (3)), though of the same order as those given by Orla-Jensen (4) for his "Betacoccus" which is identical with *S. paracitrovorus*.

It is possible that some of the acidities reported above were due to contaminants of the pasteurised milk, but no culture was used which on careful plating and microscopic examination showed contamination.

Butter-making experiments.

A series of practical butter-making experiments, designed to test the value of *S. paracitrovorus* as a starter, and its effect upon flavour, was undertaken. Three experiments were carried out at each trial.

The cream available was pasteurised at 160° F. for 20 minutes, divided into three portions, and the following cultures added:

(1) The strain of *S. paracitrovorus* to be tested.

(2) The strain of *S. paracitrovorus* to be tested + "starter."

(3) A proved, good commercial starter (as in (2)).

The six strains of *S. paracitrovorus* (A–F) were tested in this way over several months with the results shown in Table II.

The remarks in respect of flavour were made by an indep ent judge (Mr A. Todd, British Dairy Institute, Reading) who had no kn edge of the butter submitted to him.

It is clear that *S. paracitrovorus* alone can impart a good fla ur to butter, but when artificially added to a commercial starter the results w e disappoi ing in all cases but one (F).

The results shown in Table II were obtained under experi ntal condit with all precautions to avoid contamination of the pure cu es. When, h ever, a pure culture of *S. paracitrovorus* (B) was handed o to a comme dairy for use as a starter, it was found that although the bu produced by starter after the first sub-culture was excellent, its qualit ereafter was very

inferior and the use of the starter had to be discontinued. This confirmed earlier observations that *S. paracitrovorus* in pure culture is unable to hold its own against the usual starter contaminants. Efforts were therefore made to increase still further the acid producing powers of *S. paracitrovorus*.

On conclusion of the experiments detailed above a number of casein agar (Orla-Jensen (5)) stabs of the various strains of *S. paracitrovorus* were made and used over a period of a year in the attempts to raise the acid-producing properties. At the end of this time it was found that the original cultures in casein agar were still quite active and, after five months storage at 40° F., cultures in sterile milk had retained their original activity.

It was also found that in the course of experiments to determine the associative growth of *S. lactis* and *S. paracitrovorus* that when cross streaked on casein agar plates there was no inhibitory effect whatever of one upon the other.

Table II. *Flavour produced in butter by starters.*

Strain	*S. paracitrovorus* alone	*S. paracitrovorus* + commercial starter	Commercial starter alone
A	Good and pronounced	Slightly insipid	Flavour off—oily
B	Excellent flavour	Flavour good but less pronounced than with B alone	Pleasant flavour
C	Flavour good but not pronounced	Insipid—no flavour	Slightly oily—flavour not pleasant
D	Very good, clean flavour full and delicious	Good but not approaching D alone	Oily and insipid
E	Good but a little weak	Good clean full flavour, disappears quickly	Fair, slightly oily
F	Acid flavour not unpleasant but not typical	Excellent flavour one of the best	Fair—clean but insipid

The first series of experiments had shown that, always provided expert control of cultures were exercised, the flavour of butter produced by pure cultures of *S. paracitrovorus* was excellent, but no attempt was made to determine the length of time during which butter retained its fine flavour. A second series of experiments was therefore undertaken.

Second series of butter-making experiments.

Pure cultures of *S. paracitrovorus* were made by inoculation into sterile milk from the stock casein agar stabs. Starters were prepared by inoculating sterile milk with 10 per cent. of its volume of a *S. paracitrovorus* culture (strains B, D, and F), and used in the ordinary way for butter-making. The cream used was pasteurised at 160° F. for 20 minutes and cooled to 72° F. A total of samples of butter was made throughout the months of February to August. All samples were tasted by three independent judges at the time of making, and from time to time until the butter showed signs of deterioration.

Table III shows the results obtained.

It was found that as the butter-making experiments progressed the acid and flavour-producing powers of the *S. paracitrovorus* cultures diminished. Attempts were made to restore these properties.

Knudsen (6) has shown that yeast extract exerts a favourable influence upon the growth of streptococci in some milk. Beer wort which was tried and found to be successful, was added in quantities of 5, 10 and 15 c.c. to *S. paracitrovorus* cultures in 250 c.c. flasks of sterile milk. It was found that a remarkable increase in the rate of growth took place. The increase was of the same order whether 5 c.c. or larger quantities were added to the milk cultures. It was considered possible that this increase in growth rate might be due to "bios," and the *S. paracitrovorus* cultures were therefore grown by streaking on various vegetable extract agars. Grass, silage and bean agar, to which 0·5 per cent. saccharose had been added, were used.

Table III. *Score of butter made with* S. paracitrovorus *cultures and dairy starter.*

Strain	Average score (50 max.) of samples after 5 days' storage at room temp.	Average score of samples after 10 days' storage at room temp.
B	44·1	44·0
D	44·9	44·7
F	44·2	43·1
Dairy starter	42·5	43·0

Good growth of small discrete colonies, which were greyish and slightly raised, occurred on grass and silage agar. The growth on sugar-bean agar was remarkable. The tracks of the streaks were marked by a very abundant, raised, translucent, slightly greyish slimy growth—with a strong aromatic smell—which was at first taken to be a contamination. Microscopic examination showed nothing but streptococci and when the growth was sub-cultured on casein and whey agar and in milk, it gave colonies and reactions normal for *S. paracitrovorus*. Other strains of *S. paracitrovorus* behaved in a similar way when plated on sugar-bean agar. These experiments confirmed the observations of Orla-Jensen *et al.* (4) and Hucker (7).

The acidities recorded in Table I and the remarkable growth of *S. paracitrovorus* secured by the use of the media described encouraged the author to repeat the practical butter-making experiments.

Third series of butter-making experiments.

In this third series the butter was made from fresh cream pasteurised at 140° F. for 20 minutes. The cultures employed (which included two isolated locally) had been activated as described above and the same methods of preparation were employed. In this series 27 strains of *S. paracitrovorus* were used and butter was made on 16 occasions and compared on each occasion with a butter made from a commercial starter. The average acidity produced in

the ripened cream was 0·9 per cent. for commercial starters and 0·7 per cent. for *S. paracitrovorus* starters. As before, the butter was judged by independent judges who scored each sample at the end of each week for two weeks, 50 being taken as the maximum score on each occasion. The average score at the end of one week was 43·2 for *S. paracitrovorus* starters and 42·4 for commercial starters.

After two weeks the scores were 43·07 and 42·3 respectively, so that during this time the *S. paracitrovorus* butters held their flavour as well as, or better than, the commercial starters. On four occasions during the experiment, butter made with *S. paracitrovorus* starters scored 49 points out of 50, whilst the highest score with a commercial starter was 47·5 on one occasion only. It is therefore seen that, as in the previous experiments, butter of outstanding quality was obtained when *S. paracitrovorus* cultures were used, the average score being better than that of commercial starters. Nevertheless taking into consideration the length of time taken to improve the vigour and virulence of acid production, the unequal results of the butter produced and the failure to withstand more vigorously growing contaminations, it is clear that the *S. paracitrovorus* alone is unsuitable for use as a pure culture starter.

SUMMARY.

The experiments have shown that although butter of exceptionally fine flavour can be produced by the use of pure culture starters of *S. paracitrovorus* under laboratory control, the uncertainties under practical conditions, due to its weak growth, are too marked to warrant its general use. Both *S. citrovorus* and *S. paracitrovorus* are unable to compete successfully with the inevitable contaminants encountered in practice. The value of vegetable media such as grass, silage and bean agar for growing streptococci such as *S. paracitrovorus* has been confirmed, but as observed by Orla-Jensen *et al.*(4) the subsequent growth in milk media lacks the vigour which might be expected.

REFERENCES.

(1) ROGERS, L. A. and GREY, C. E. (1909). *U.S. Dept. Agr. Bull.* **114**, Bur. Anim. Ind.
(2) HAMMER, B. W. (1920). *Iowa Agric. Exp. Sta. Res. Bull.* **63**.
(3) —— (1923). *Ibid.* **81**.
(4) ORLA-JENSEN, S., ORLA-JENSEN, A. D. and SPUR, B. (1926). *J. Bact.* p. 333.
(5) ORLA-JENSEN (1919). *The lactic acid bacteria*, Copenhagen.
(6) KNUDSEN, S. (1927). *Cbl. f. Bakt.* Abt. II. **71**, 500.
(7) HUCKER, G. J. (1929). *Cbl. f. Bakt.* Orig. 111, i–iii, 31–64.

ABSTRACTS AND REVIEWS

BACTERIOLOGY

General

S. E. Owen. **The relation of media pH to the bacteriostatic action of dyes.** *Amer. J. Pharm.* **102**, 154–8, 1930. (*Chem. Abst.* **24**, x, 2488, May 20, 1930.)

The *p*H value of media is an insignificant factor in the selective action of gentian violet, basic fuchsin and perhaps also methylene blue. Eight of the certified food dyes tested show no bacteriostatic action at *p*H values ranging from 5·4 to 10·4. Guinea green inhibits the growth of *B. anthracis* in the acid range and at *p*H 7·4 but not in other alkaline *p*H values greater than this. Erythrosine inhibits the growth of *B. anthracis* at all *p*H values studied. A brief bibliography and also a comprehensive reference list on the bacteriological importance of stains, dyes and chemicals are appended.

A. Guilliermond. **Sur la toxicité des colorants vitaux.** (Toxicity of vital stains.) *C. R. Soc. Biol.* **104**, xix, 468–72, June 6, 1930.

The toxicity of various vital stains for *Oidium lactis* and other fungi (Mucoraceae, *Spermophtora gossypii*, *Penicillium glaucum* and various yeasts) has been determined and compared with their toxicity for *Saprolegnia*. It was found that for each one there was a limiting dose which varied with the fungus and the culture medium, and was much lower for *Saprolegnia* than for the other fungi. The latter are not coloured during their growth in media containing the stains, neutral red included, but *Saprolegnia* accumulates neutral red within its cells. E. R. Hiscox

C. H. Werkman. **An improved technic for the Voges-Proskauer test.** *J. Bact.* **20**, ii, 121–6, August 1930.

After discussing the chemical mechanism of the test and the use of oxidising agents such as H_2O_2 to hasten the appearance of colour, the author describes the method of using Fe_2Cl_6 as a catalyst.

Two drops of 2 per cent. solution of Fe_2Cl_6 are added to 5 c.c. of the culture followed by shaking with 5 c.c. of 10 per cent. solution of NaOH. In positive tests a bright copper colour, which is stable, is formed at once. A. T. R. Mattick

F. C. Happold. **The correlation of the oxidation of certain phenols and of dimethyl-*p*-phenylenediamine by bacterial suspensions.** *Biochem. J.* **24**, vi, 1737–1743, 1930.

Strict aerobes oxidise dimethyl-*p*-phenylenediamine, but this oxidation may be masked by reducing systems present. It has been shown that tyrosinase in the presence of atmospheric oxygen and certain phenols which form *o*-quinones, causes the deamination and decarboxylation of amino acids thus forming an aldehyde, ammonia and carbon dioxide.

The author has investigated the relationships between these reactions in the case of a number of bacteria.

All organisms giving the dimethyl-*p*-phenylenediamine reaction catalyse the oxidation of catechol to *o*-benzoquinone. The enzyme responsible is heat labile. None of the organisms tested (except a tyrosinase-containing *Vibrio*) could deaminate an

ERRATA

VOLUME II, No. 2.

p. 209, Fischer (second line)

for paratyphus *read* paratyphoid

amino acid in the presence of a monohydric phenol, although those producing a colour change with catechol could deaminate in the presence of this phenol.

Only the aerobic spore-formers catalysed the oxidation of guaiacol to a red brown substance. J. G. Davis

O. Bier and J. Reis. **La réaction oxydante directe chez les streptocoques.** (Direct oxidising reaction of the streptococci.) *C. R. Soc. Biol.* **104**, xxi, 707–8, June 20, 1930.

The authors have examined cultures of 110 streptococci of very varied origin, and have observed that, contrary to previous statements, these organisms did not invariably give negative results, but that 23·6 per cent. gave a typical oxidising reaction. From this it follows that the reaction cannot be used as a means of distinguishing gonococcus and meningococcus from other cocci. E. R. Hiscox

G. Fischer. **La croissance de B. coli et des bacilles paratyphiques dans des milieux demisolides.** (Growth of *B. coli* and paratyphus bacilli in semi-solid media.) *C. R. Soc. Biol.* **104**, xix, 528–30, June 6, 1930.

When a culture of *B. coli* is inoculated into a medium containing 2 per cent. peptone and 0·2 per cent. agar (*p*H 7·5), there is normal development at the surface to a depth of 1 cm. Below this there is a series of horizontal rings, 0·2–2 mm. thick, with clear areas between. A similar effect is obtained in Petri dishes, but the rings are concentric. The growth in the horizontal and concentric ring consists of long, thin, mobile bacilli, whilst the organisms in the surface growth are short, thick, non-mobile rods, similar to the normal forms of *B. coli*.

If the medium is heated and re-inoculated it will not support growth. If a layer of fresh medium is poured on the surface, growth will not occur at the bottom of this layer. It would appear that the exhaustion of the medium is due to the development of toxins and not to impoverishment. E. R. Hiscox

H. Hoek. **Bacterial filtration by the Berkefeld filter and the size of the pores.** *Chem. Fabr.* pp. 249–50, 1930. (*Brit. Chem. Abst.*, p. 743, August 15, 1930.)

For the purpose of bacterial filtration by cylinders the size of the largest pores is the most important factor to be considered. This can be measured by noting that pressure at which air bubbles begin to be forced through the cylinder when the latter is held under water.

An efficient bacterial filter should not pass air bubbles under a pressure of 0·55 atmospheres. J. G. Davis

H. Plotz and J. Geloso. **Relations entre la croissance des microorganismes anaérobies et le potentiel du milieu de culture.** (Relation between growth of anaerobic micro-organisms and the potential of the culture medium.) *Ann. Inst. Pasteur*, **45**, v, 613–40, May 1930.

Previous workers have reported that anaerobes can only grow over an *r*H range of 0 to 14. The authors have employed an ingenious method of measuring potentials *in vacuo*, employing an H-tube one arm of which contains a saturated calomel electrode and the other the culture medium. Contact is made between the solutions through the minute layer of adsorbed electrolyte on the glass. E_h values obtained with different cultures of anaerobes in this apparatus are shown graphically, anaerobiosis being obtained either by evacuating or by the addition of the usual reducing substances (alkaline-heated glucose, cysteine, sodium hydrosulphite, titanium citrate, glucose and platinum black). *Cl. tetani*, *E. coli* and *Cl. botulinum*, *bifermentans*, *putrificum*, *sporogenes*, *oedematis-maligni* and *histolyticum* were studied in this way. All tended finally to give an E_h of − 0·265 (*r*H = 5·5). Determinations on unino-

culated broths showed that, in the presence of the reductants listed above, the potentials verged towards this value (*r*H 7·5). Thus the organism has on the medium an effect similar to that of platinum black, *i.e.* that of catalysing the dehydrogenation of the sugars. *Cl. tetani* was found to grow only at an *r*H range of 0 to 14.

J. G. Davis

G. Tanman. **The poisonous action of some metals and alloys on bacteria.** *Forschungen u. Fortschritte*, **5**, 257, 1929. (*Chem. Abst.* **24**, xiv, 3529, July 20, 1930.)

Copper, nickel, silver and cobalt in a culture of bacteria exert a toxic action in extremely low concentrations (oligodynamic action).

Manganese, aluminium and iron do not appear to be poisonous under similar conditions. Results obtained with alloys are also given. (Cf. Richet, *Comp. Rend.* **114**, 1494.) J. G. Davis

J. P. Scott. **A simple mineral mixture agar for the cultivation of Pasteurella boviseptica.** *J. Bact.* **20**, i, 9–14, July 1930.

Ions essential for the growth of anaerobes in protein media are potassium, ammonium, dihydrogen phosphate, bicarbonate and pyruvate.

The effect of different concentrations of salts on the growth of *P. boviseptica* is described and a salt mixture evolved, which when added to a protein medium increases the growth, viability and pathogenicity of this organism. Viability is better at room temperature than at 50° C. or less. Gas formation by *Pasteurella* organisms is increased by adding certain salts to the medium. J. G. Davis

O. Klingmüller. **Untersuchungen über Unterschiede des Streptococcus cremoris und Streptococcus mastitidis.** (The differences between *Streptococcus cremoris* and *Streptococcus mastitidis*.) *Milchw. Forsch.* **10**, v–vi, 431–54, October 1930.

The investigation of the morphological and physiological characters of a number of strains of *Str. cremoris* and *Str. mastitidis* confirmed the view that the two species were very much alike. A close relationship was indicated by their morphology and by the facts that they produced equal quantities of acid in milk, brought about a similar degradation of casein, were both able to produce gas from lactose and dextrose and gave negative tests for bacteriophage, whilst *Str. mastitidis* rapidly lost its pathogenicity. On the other hand, the difference in their resistance to high temperatures and the longer life of *Str. mastitidis* argued against such a relationship. If the view be taken that *Str. cremoris* is a race of *Str. mastitidis* it is easy to understand that it will be more sensitive to external influences. E. R. Hiscox

C. A. Magoon and B. C. Brunstetter. **A new vessel for the efficient aeration of bacterial cultures in liquid media.** *J. Bact.* **19**, vi, 415–22, June 1930.

This paper gives a description of an ingenious culture vessel designed to secure uniform conditions of food supply and aeration for studying the physiology of micro-organisms. A. T. R. Mattick

F. Krasnow and M. Reiner. **Biochemical studies of streptococci. VI. Relation of osmotic pressure to availability of synthetic media.** *J. Dental Res.* **9**, 733–40, 1929. (*Chem. Abst.* **24**, xi, 2773, June 10, 1930.)

The 46 media used exhibited a wide range in osmotic pressure; the lowest pressure was less than 50 mm. Hg, the highest 915 mm. The number of media showing growth increased as the osmotic pressure decreased.

p. 211, Todd (last line)
for virulent *read* avirulent

P. Mazé and P.-J. Mazé. **L'action de l'oxygène libre sur les ferments lactiques se développant en anaérobiose.** (The action of free oxygen on the lactic ferments developing under anaerobic conditions.) *C. R. Soc. Biol.* **105**, xxxii, 519–20, November 28, 1930.

The presence of a sterile zone at the limit of the regions of aerobic and anaerobic life of a culture of a lactic ferment allows of the demonstration of the method by which free oxygen inhibits the evolution of anaerobic micro-organisms. The products of the anaerobic fermentation are transformed by the oxygen which diffuses through the medium into antiseptics which destroy the organisms in that zone where these compounds are abundant. It has been demonstrated that acetaldehyde is produced in this region though probably this is not the only reaction which occurs.

E. R. Hiscox

G. S. Graham-Smith. **The longevity of dry spores of B. anthracis.** *J. Hygiene*, **30**, ii, 213–5, June 1930.

Material containing numerous young spores of *B. anthracis* was inoculated on small pieces of dry sterile canvas, contained in Petri dishes which were stored in a cupboard with glass doors. The spores were thus kept dry at room temperature and exposed to diffuse daylight. At intervals pieces of cloth were removed and examined for the presence of living spores. Within a few months about 50 per cent. of the spores appeared to have lost their power of germinating, but of the remainder, a considerable proportion were still capable of germination after 10 years. The proportion of living spores steadily decreased until they all apparently disappeared in about 23 years.

E. R. Hiscox

E. W. Todd. **Virulence of haemolytic streptococci. I. The influence of oxygen on the production of glossy variants.** *J. Exp. Path.* **11**, v, October 1930.

By long subcultivation on aerobic agar, glossy (smooth) colonies of avirulent strains may be obtained. These are more resistant to peroxide than the virulent (rough) strains; the latter also produce more peroxide than the avirulent strains and so suffer auto-sterilisation earlier. The addition of hydrogen peroxide accelerates the selection of smooth types, while catalase prevents the appearance of virulent types.

See Errata

J. G. Davis

G. A. Hunt and L. F. Rettger. **A comparative study of members of the lactobacillus genus, with special emphasis on lactobacilli of soil and grain.** *J. Bact.* **20**, i, 61–81, July 1930.

A brief review of previous work on the specification of the lactobacilli is followed by a description of the morphological and cultural characteristics of 36 strains either isolated from soil, grains and faeces, or from stock cultures. Growth was increased by the addition of yeast or tomato extract. The sugar fermentations and serological relationships are described in detail; these do not always confirm each other. Cell morphology and colony formation serve only as preliminary means of identification. Soil organisms do not usually ferment lactose, *L. bulgaricus*, *acidophilus* and *odontolyticus* do. Gas-producing strains rapidly lose this property when cultured in the laboratory. It is interesting to note that although grains protected from animal (mice) contamination rapidly ceased to yield lactobacilli, all attempts to isolate lactobacilli from the intestinal contents of mice failed.

J. G. Davis

W. Loele. **Relation of the oxidising substances in bacteria and yeasts to cellular oxidases.** *Arch. path. Anat.* (*Virchows*), **267**, 733–45. (*Chem. Abst.* **24**, x, 2488, May 20, 1930.)

Naphthol and benzidine-oxidases are found in yeasts; the appearance of the reaction is dependent on the nutrient medium and on the age of the culture. Certain groups of bacteria give the oxidase reaction.

E. W. and A. E. Stearn. **Differential action of oxidising agents on certain Gram-positive and Gram-negative organisms.** *J. Inf. Dis.* **46**, vi, 500–13, June 1930.

Gram-negative organisms are more susceptible to oxidising agents than Gram-positive organisms. A few Gram-negative organisms, however, approach the Gram-positive in their resistance to such reagents. The mechanism of this inhibition differs from that of ordinary dyes. These results are discussed in relation to modern ideas on oxidation-reduction environment. J. G. Davis

E. Lepper and C. J. Martin. **The oxidation-reduction potential of cooked-meat media.** *J. Exp. Path.* **11**, iii, 137–39, June 1930.

The oxidation-reduction potential of meat media under conditions in which these are used for the cultivation of anaerobes is of the order of − 0·2 volt. Discrepancies between gold and iridium electrodes did not exceed 30 millivolts. This value is consistent with the estimated values of the system parahaematin-haemochromogen which is alleged to be responsible for the equilibrium attained in sterile cooked-meat media. J. G. Davis

E. Lepper and C. J. Martin. **The oxidation-reduction potential of cooked-meat media following the inoculation of bacteria.** *J. Exp. Path.* **11**, iii, 140–45, June 1930.

E_h curves of some facultative and strict anaerobes are given. The effect of the hydrogen evolved in the solution upon the electrode readings, and the chemical mechanisms involved in the poising of the oxidation-reduction potential are discussed. J. G. Davis

L. F. Hewitt. **Oxidation-reduction potentials of cultures of haemolytic streptococci.** *Biochem. J.* **24**, ii, 512–24, 1930.

This paper gives the oxidation-reduction potential curves of cultures of haemolytic streptococci in various media and under various conditions of oxygen tension. The author claims that "streptococci" are not favoured by low reducing potentials although his evidence is based on the behaviour of only one strain. Serum proteins inhibit the fall of potential in aerobic cultures. This effect is ascribed to the oxygen carrying capacity of sulphur groups in the molecule. Peroxide is frequently formed in aerated cultures (E_h + 0·5 and rH 30). Low concentrations (0·007 per cent.) were not toxic. Aerated cultures gave an rH of 17–21 and freshly boiled media cultures an rH of 9–13. J. G. Davis

L. F. Hewitt. **Oxidation-reduction potentials of staphylococci cultures.** *Biochem. J.* **24**, iii, 676–81, 1930.

E_h curves for cultures of *Staphylococcus aureus* under various conditions of oxygen tension and media are given. This organism resembles *C. diphtheriae* in maintaining intense reducing conditions in the presence of oxygen, and also in maintaining this level for a long period. Glucose broth does not give the low values obtained with ordinary broth. [This author does not give values for uninoculated media or proof of viability during the experiments. It is possible, therefore, that some of the values given are due to the medium rather than to the organisms.] J. G. Davis

L. F. Hewitt. **Oxidation-reduction potentials of cultures of C. diphtheriae. I.** *Biochem. J.* **24**, iii, 669–75, 1930.

C. diphtheriae produces a higher reducing intensity than the haemolytic streptococci. Both types, however, show a more rapid fall in potential under aerobic conditions than when sealed. The rise in potential subsequent to the logarithmic growth rate period obtained with the streptococci does not occur with *C. diphtheriae*. Serum possesses the same oxygen-carrying effect that was observed with the streptococci.

J. G. Davis

J. A. Cranston and B. Lloyd. **Studies in gas production by bacteria. I. Apparatus for the measurement of the rate of gas production. II. Denitrification and bacterial growth phases.** *Biochem. J.* **24**, ii, 525–8, 529–48, 1930.

I. The author describes three simple methods which have been devised for the measurement of gas evolution by bacteria: (1) a pyknometer method for use where compactness is required; (2) a capillary tube method for making simultaneous readings of a large number of cultures; and (3) a large-scale apparatus in which the gases can be analysed and samples of the medium withdrawn.

II. Experiments are described which show the relationship of the rate of gas production to the growth rate. From the study of the effect of lag in cultures of nitrate and nitrite broth the authors suggest the following scheme for denitrification:

$$KNO_3 \text{ and } KNO_2 \rightarrow K_2N_2O_2 \rightarrow K_2O + N_2.$$

J. G. Davis

G. S. Wilson. **The growth of Br. abortus in sealed tubes.** *J. Exp. Path.* **11**, iii, 157–63, June 1930.

The efficacy of the well-known method of growing *Br. abortus* is due to the production of CO_2 by the sealing of the tubes (flaming the cotton-wool, melting the sealing wax, etc.). A tube which has been sealed in this way may contain 1 to 3 per cent. CO_2.

J. G. Davis

L. H. Lampitt. **Research work in the yeast field.** *J. Inst. Brewing*, **3**, vi, 250–60, June 1930.

A review of the researches on yeasts from the time of Pasteur until the present day.

E. R. Hiscox

F. Marboe. **Ueber den Einfluss blanker Metalle auf Hefe.** (Effect of bright metals on yeast.) *Zbl. f. Bakt.* II, **81**, i–vii, 67–73, May 1930.

The action of a metal depends on its specific activity in conjunction with its solubility. The boiling of a medium containing metal reduces its action. The influence of the metals appears to depend on the toxic action of metal salts and on their reducing and oxidising properties.

E. R. Hiscox

H. Tamiya and T. Hida. **Vergleichende Studien über die Säurebildung, die Atmung, die Oxydasereaktion und das Dehydrierungsvermögen von Aspergillusarten.** (Acid formation, respiration, oxidase reaction and dehydrogenating power of species of *Aspergillus*.) *Acta Phytochimica*, **4**, 343–61, 1929. (*Zbl. f. Bakt.* II, **80**, xxiii–xxvi, 499, May 1930.)

G. Behr. **Über Autolyse bei Aspergillus niger.** (The autolysis of *Aspergillus niger*.) *Arch. f. Mikrobiol.* **1**, iii, 418–9, September 1930.

The author distinguishes two forms of autolysis by *A. niger*. The first occurs at about *p*H 1 ("acid" autolysis) and liberates organic-nitrogen containing compounds. In addition a yellow pigment is formed, the chitin content increases progressively

and the decrease in mycelial growth is relatively small. The second or "neutral" autolysis occurs at a *p*H of about 6·5. The absence of organic-nitrogen containing compounds, the formation of ammonia, a violet pigment and a humin-like substance, the relatively great mycelial atrophy and the complete disappearance of chitin are characteristic of this mode of autolysis.

The investigation gave no evidence that organic acids originated by deamination. Some properties of the chitin are described. J. G. Davis

Dairy Bacteriology

L. Vogeler. **Über sporenbildende Bakterien im Darmkot des gesunden Rindes unter der Berücksichtigung ihrer Bedeutung für die Milchwirtschaft.** (Spore-forming bacteria in the faeces of healthy cattle with reference to their importance for the dairy industry.) *Milchw. Forsch.* **10**, iii–iv, 180–230, July 1930.

The author has made a qualitative and quantitative study of the spore-forming bacteria in the faeces of healthy cows during different periods of feeding. During summer feeding in the field, the total number of bacteria and the percentage of spore-formers are much lower than during the period of stall feeding. During the former the *mesentericus* group is present in much larger numbers than the *mycoides*, although these two groups form the bulk of the spore-bearing bacteria. The *subtilis* group is rarely present. In the latter period the *mycoides* group is more abundant than the *mesentericus* and the *subtilis* group is frequently found though not in large numbers.

The various spore-forming bacteria are described and identified and their resistance to heat, their temperatures of growth, their reaction to salt, and acid-tolerance are determined, and correlated with their influence on products made from milk containing them. E. R. Hiscox

P. Mazé and P.-J. Mazé. **Les substances réductrices du lait assurent le développement prédominant de la fermentation lactique dans le lait.** (The reducing substances in milk assure the predominance of lactic fermentation in milk.) *C. R. Soc. Biol.* **105**, xxxii, 518–9, November 28, 1930.

The predominance of lactic ferments in milk which has soured naturally is due to the presence in the milk of reducing substances capable of fixing atomic hydrogen in an acid medium. When an agar stab is heavily inoculated with a young culture of a lactic ferment, colonies develop at the top and bottom of the tube, but between these two areas there is frequently a sterile zone of variable thickness, the formation of which depends on the species of ferment and the nature of the medium. If small quantities of methylene blue are added to the medium the sterile zone increases in thickness and tends to approach the surface. The methylene blue favours the anaerobiosis of the lactic ferment since it acts as an acceptor of nascent hydrogen and furnishes indirectly oxygen for anaerobic respiration. The richer the medium is in hydrogen acceptors in the presence of free acid the more will it favour the anaerobic development of lactic ferments. E. R. Hiscox

G. Koestler. **Euterbakterien und Milchsekretion.** (Udder bacteria and milk secretion.) *Schweiz. Milchztg.* No. 29, 197, 1930. (*Milchw. Zbl.* **59**, ix, 140, May 15, 1930.)

Udder bacteria can exert by their metabolic products a stimulus in certain parts of the milk gland. The disturbance so caused in milk production gives a secretion whose physico-chemical properties always show the same type of variation from the usual secretion. Such a milk possesses all the characteristics of so-called salty

(rässsalzige) milk. The author concludes that the strength of the reaction, *i.e.* the degree to which the salty characters are expressed, depends upon three factors: (1) the kind of micro-organism, (2) the number of bacteria present in the udder, and (3) the individuality of the milk gland. M. I. CHRISTIAN

H. BARKWORTH. **Some factors affecting the keeping quality of milk.** *Milk Industry*, **10**, xii, 65–9, June 1930.

The paper is a resumé of the results of 3167 keeping-quality tests carried out on morning's milk. Under similar conditions after arrival at the laboratory the keeping quality of a sample of milk is related to the bacterial count rather than the temperature on arrival. Coliform organisms depress the keeping quality of milk. The fat content of milk has little if any influence on its subsequent keeping quality.

A table is set out giving the variations in keeping quality on a basis of total count. The range of variations is approximately a total of 24 hours, 12 hours above or below the average for samples of any particular bacterial count. Two-thirds of the samples fall within these limits of variation. L. J. MEANWELL

S. B. THOMAS. **A preliminary report on the bacteriological examination of fresh cream and artificial (reconstituted) cream.** *Welsh J. Agric.* **6**, 284–9, 1930.

Sixty-two samples of fresh cream and reconstituted cream have been examined. The plate count of the former was found to be about 40 times that of the latter. Aerobic spore-formers were found in the artificial cream. The average keeping quality of the artificial cream was 56 hours, of the fresh cream 38 hours. While the fresh cream developed a "sour" taint, a butyric or putrefactive odour was noticed in the artificial cream. The average reduction time of methylene blue for the fresh cream was 2 hours, for the reconstituted material 8·2 hours. Anaerobic spore-formers were found in half the artificial cream, while 75 per cent. of the milk powders used in their manufacture contained these organisms.

(The importance of this last finding from the point of view of infection of other dairy produce, *e.g.* starter and cheese, must be considered.) J. G. DAVIS

B. W. HAMMER and V. H. PATIL. **Proteolysis by Streptococcus lactis, with special reference to butter cultures and butter.** *Iowa Res. Bull.* **123**, March 1930.

The first part of this bulletin is devoted to a review of the literature upon proteolysis by *S. lactis*.

The original work reported was carried out upon about 120 strains of *S. lactis* isolated from milk, sweet and sour cream and from butter cultures by plating on whey agar and fishing colonies. Proteolysis was measured by determination in milk cultures of the soluble and amino nitrogen. In butter protein decomposition was followed by similar determinations upon the filtrate from the non-fatty material of the butter. Incubation was carried out at room temperature (presumably about 23° C.).

A description of the chemical methods employed is given. In one series it was found that appreciable proteolysis in milk occurred in as short a time as 7 days without the addition of $CaCO_3$. The more rapid acid producers gave the largest amount of proteolysis. In a further series proteolysis was appreciable in 36 hours. Tests with butter cultures showed them to be actively proteolytic in sterile and in pasteurised milk. Proteolysis was evident shortly after coagulation had taken place.

The organisms associated with *S. lactis* (*S. citrovorus* and *S. paracitrovorus*) in starter appeared to be practically non-proteolytic.

The lactic acid produced in butter cultures was not responsible for proteolysis. Cultures of *S. lactis* fell into two groups one of which coagulated milk quickly and

caused proteolysis, the other being variable in its rate of coagulation and non-proteolytic. Experiments to determine the influence of temperature in proteolysis showed it to be unaffected but coagulation was more rapid at higher temperatures (30° to 37° C.) than at room temperature.

When butter was examined for proteolysis it was found that *S. lactis* had no influence. On storage slight proteolysis took place which could not be referred to *S. lactis*. A. T. R. MATTICK

K. VAS. **Mykologische Untersuchung der Butter mittels Mikroskop in erwärmten und Oelpräparaten.** (Mycological examination of butter by means of the microscope in heated and oil preparations.) *Kiserl. Közlemények*, **32**, 23, 1929. (*Milchw. Forsch.* **10**, i–ii, Ref. p. 39, May 1930.)

Quick methods for the microscopic examination of butter are suggested. These methods effect transparency of the butter samples either by melting or solution in oil while stains are applied in alcoholic or ethereal solution. W. L. DAVIES

C. A. MITHCHELL and R. C. DUTHIE. **Note on tubercle bacilli of avian origin harboured in the udder of a cow.** *Canad. J. Res.* **2**, vi, 406, June 1930.

Two hundred and ten days after injecting intravenously a previously tubercle-free cow with tubercle bacilli from a tubercular cow, the udder was removed and fragments injected into a guinea-pig and chicken. The chicken was positive to avian and bovine tuberculin 95 days after. The guinea-pig was negative, killed after 45 days. The chicken, killed 106 days after, showed progressive tuberculosis. Material from the chicken was inoculated into a guinea-pig, chicken and rabbit. The chicken died 120 days after and material from it was injected into another chicken, rabbit and guinea-pig. This rabbit was negative 109 days after. The chicken and guinea-pig reacted to avian and bovine tuberculin 52 and 46 days after respectively.

C. MADDOCK

CHEMISTRY (ORGANIC, INORGANIC AND PHYSICAL)

DAIRY CHEMISTRY

A. F. LERRIGO. **The routine detection of nitrates in milk.** *Analyst*, **55**, 652, 433–5, July 1930.

A modified technique for the diphenylamine test for nitrates in milk is described which is capable of detecting nitric nitrogen in the proportion of 0·1 part nitric nitrogen per 100,000. Special precautions are necessary to ensure absence of nitrates from the reagents used. In practice the addition of 5 per cent. water to milk can be detected if the water added contains 0·5 part nitric nitrogen per 100,000 and this without concentrating the milk serum.

Of 102 samples which contained less than 8·5 per cent. solids-not-fat, 50 gave a positive reaction for nitrate. Nitrates have also been detected in cream and the test might, it is suggested, afford additional evidence as to the artificial nature of cream.

J. GOLDING

K. VAS. **Die Löslichkeit von Kupfer und Zink in Milch und Molke.** (Solubility of copper and zinc in milk and whey.) *Kiserl. Közlemények*, **26**, 1, 1923. (*Milchw. Forsch.* **10**, i–ii, Ref. p. 17, May 1930.)

The rates of solubility of zinc and copper in fresh and increasingly acid samples of milk and whey have been studied. In fresh milk 0·75–2·25 mg. of zinc per square decimetre are dissolved in 48 hours, 1·25 mg. of copper per square decimetre being the amount of copper dissolved in the same time. Soured milk and whey are

much more active on zinc plates (dissolving 20·75 mg. per dm.^{2}). The nature of the surface was found to be important, old and rough surfaces being found to be more easily acted on. Polished surfaces of copper resisted corrosion more than rusted (verdigris) copper surfaces. Thirty times more copper was found to dissolve in both fresh and sour milk and whey by not polishing the metallic surface. The importance of these findings to the dairy industry is stressed. W. L. DAVIES

W. LIEBSCHER. **The influence of the addition of a mineral salt mixture to feed on the quantity and some constituents of milk.** *Landw. Vers. Sta.* **109**, 347–62, 1929. (*Chem. Abst.* **24**, x, 2509, May 20, 1930.)

The addition of 38 g. of CaO, 16 g. of P_2O_5 and 4·75 mg. of I (as NaI) in the form of a mineral salt mixture "Ancora (F)" to the control feed gave the following results: The milk yield from 7 cows was not influenced; with one cow there was an influence. Neither in the percentage nor in the weight of the fat or dry matter of the milk were there any changes which could be correlated with certainty with the mineral salt addition. The specific gravity and the fat-free dry matter were somewhat increased. The mineral salt accordingly seems to exert a slight influence in a positive sense on the milk constituents which determine the specific gravity and the fat-free dry matter. No influence on the live weight could be established. The increase of individual ash constituents in the feed was therefore without special effect in the present case, and would, in all probability, have no effect or only a slight influence when green feed or pasture in summer, or good clover and meadow hay in winter, with average milk production, are the basis of nutrition.

A. DÜRING. **Comparative sucrose determinations in sweetened condensed milk.** *Pharm. Zentr.* **71**, 49–51, 1930. (*Brit. Chem. Abst.* B, p. 437, May 16, 1930.)

Comparison of the official method (Röttger, 1926, **1**, 5th ed. 407) with the modified method described below showed the latter to be the more rapid, convenient, and accurate. The condensed milk (10 g.) is mixed with 60 c.c. of hot water and treated with a suspension of 1 g. of calcium oxide in a little water. The mixture is shaken, heated on the water bath at 80° C. for 1 hour, cooled, and treated successively with 10 c.c. of dilute sulphuric acid (1 : 4), 5 c.c. of lead acetate, and 5 c.c. of saturated sodium phosphate solution. After the precipitate has settled, the liquid is filtered by suction through a porcelain funnel covered with cloth and a thin layer of asbestos, and the residue on the filter is washed until free from acid. The filtrate is made up to 200 c.c., treated with 0·5 g. of ignited charcoal powder, and again filtered. The rotation of the solution in a 200 mm. tube at 20° C. (measured in the Laurent polarimeter) multiplied by 15 gives the weight of sucrose in 100 g. of sample.

C. W. TURNER and A. C. HASKELL. **A statistical study of the size of fat globules in cow's milk.** *Missouri Res. Bull.* **130**, November 1929.

Fresh milk was obtained for these studies from the dairy herd of the Missouri Experiment Station. The milk was treated with a glycerin-cochineal solution before mounting on a slide for examination. Micro-photographs with a magnification of 1000 were taken; from these the size of the globules was measured. J. GOLDING

M. B. MACDONALD, E. C. ANDES and F. A. BRIGGS. **The effect of mineral oil treatment on the composition of milk.** *J. Home Econ.* **22**, 213–8, 1930. (*Chem. Abst.* **24**, x, 2508, May 20, 1930.)

Addition of mineral oil to milk to remove the "onion flavour" does not affect its butter-making properties. The butter fat absorbs less than 1 per cent. mineral oil, and less than 0·5 per cent. butter fat is lost. The $SbCl_3$ test showed no detectable loss of vitamin A.

H. R. Whitehead. **The reduction of methylene blue in milk. The influence of light.** *Biochem. J.* **24**, 579–84, 1930.

Methylene blue added to fresh milk of good quality is reduced in a short time in the presence of sunlight at 37° C. No bleaching occurs in darkness at the same temperature. An enzyme cause is ruled out by the occurrence of bleaching under the same conditions in milk heated at 100° C. for half-an-hour. Fat-free milk does not give the reaction, but sodium oleate added to such milk restores the property to bleach methylene blue, whereas the addition of sodium palmitate does not. It is suggested that sunlight catalyses an oxidation-reduction reaction in which unsaturated fats are oxidised and methylene blue is reduced.

(No reference is made to the previous work of Greenbank and Holm (*Ind. Eng. Chem.* (anal. ed.), **2**, ix, 1930) which embodies the same principle.) W. L. Davies

M. B. Macdonald and A. Glaser. **The removal of bitter flavour from "bitterweed" cream.** *Tennessee Circ.* **26**, 1929. (*Chem. Abst.* **24**, x, 2508, May 20, 1930.)

If cows eat the bitterweed (*Helenium tenuifolium*) or common yellow dog fennel, a bitter taste is imparted to milk. The bitter substance was found in both fresh and cured bitterweed. It is a crystalline, non-volatile, nearly colourless, odourless, and very bitter substance. It is insoluble in fat and mineral oil but soluble in H_2O, and for this reason is in solution in the milk serum. The bitter substance could only be removed from milk by several washings and separations in a separator; thus cream could be rendered practically free from flavour.

P. Sannyal. **Recherche de la falsification du beurre par les graisses animales et végétales.** (Adulteration of butter with animal and vegetable fats.) *Mem. Dept. Agric. India*, **10**, 153–5, 1929. (*Le Lait*, **10**, xcv, 562, May 1930.)

One gram of melted butter is dissolved in 3 ml. of dry ethyl acetate at 30° C. 4 ml. of 93 per cent. alcohol are added and the mixture kept at 30° C. for half-an-hour. If foreign fats are absent, no precipitate appears. A precipitate forms with the minimum of 5 per cent. added animal fat or 12 per cent. added vegetable fat. Butter from cows fed on cottonseed cake gives a positive result and the test is therefore modified by dissolving the butter (1 g.) in 4 ml. of ethylacetate. The test is thus rendered less sensitive for the detection of added vegetable fat. W. L. Davies

S. B. Thomas. **Laboratory work in connection with butter competitions.** *Welsh J. Agric.* **6**, 279–84, 1930.

Laboratory work in connection with farm-butter competitions consisted of a keeping-quality test, estimations of bacterial and mould and yeast counts, a bottle incubation test and a colorimetric estimation of hydrogen-ion concentration. In the keeping-quality test, unsalted butter was kept at 60° F., the number of days taken to develop a taint sufficient for it to be condemned for consumption being the index of the keeping quality. The keeping quality varied from 5 to 21 days, the averages for the various competitions varying from 8 to 13 days. W. L. Davies

N. King. **Zur Kenntnis der kontinuierlichen Phase der Butter.** (The continuous phase of butter.) *Milchw. Forsch.* **10**, i–ii, 68–71, May 1930.

Microscopic examination of the structure of butter by the "continuous phase dilution method" revealed the continuous phase in butter to be the butterfat. The method consisted in diluting the "droplet concentration" by a centrifugal method, the form and physical properties of the water droplets and protected fat globules being observed under conditions which virtually amounted to dilution of the continuous phase with pure butterfat. W. L. Davies

N. KING. **Kolloidchemie der Butter. Ein Beitrag zur Histologie der technischen Stoffe.** (The colloid chemistry of butter. A contribution to the series "structure of technical materials.") *Koll.-Zeit.* **52**, 319-32, 1930.

The relation between the physical structure of butter and its properties with special reference to chemical and bacteriological changes is described.

The three theories of the structure of butter (*a*) emulsion of water in fat (Fischer), (*b*) fat in water emulsion (Rahn), and (*c*) water droplets and fat globules in fat (King) are discussed. Fat exists in two forms in butter, namely, as fat globules surrounded by a protein coating, and a continuous phase of fat. The significance of the continuous phase as a water-holding medium is referred to. The composition and distribution of the constituents in the aqueous phase are discussed. The functions of the water-fat and the air-fat interfaces during the chemical and bacteriological changes occurring in butter are explained. W. L. DAVIES

H. RUEBENBAUER. **Analysis of sheep's milk cheese produced in the Tatra mountains.** *Przemysl Chem.* **14**, 169, 1930. (*Brit. Chem. Abst.* B, p. 528, June 13, 1930.)

The fat content of milk of sheep bred in the Tatra mountains varies from 6·86 per cent. in May to 9·12 per cent. in August, whilst the content of solids-not-fat remains constant (10·99–11·33 per cent.). An analysis of cheese made from the milk gives (per cent.): water content 36·81, fat 33·5, ash 4·54 (including 2·47 per cent. NaCl), casein 15·17, albumin 1·32, amino compounds 6·69, diamino compounds 0·72, ammonia 0·08, and lactose 1·17 per cent. The fat has M.P. 28·2°; F.P. 18·9°; critical dissolution temperature (alcohol) 53°; refractivity 44·1°; acidity corresponding with a free oleic acid content of 0·7191 per cent.; saponification value 229·1, soluble and insoluble volatile acid value, respectively, 28·2, 3·8; non-volatile acid content 86·4 per cent.; iodine value 35·2; thiocyanogen value 35·2; octoic acid value 2·4; lauric, stearic and oleic acid contents respectively 6·95, 4·7, 38·2 per cent.; palmitic and myristic acid content 33 per cent.; unsaponifiable residue 1·8 per cent. The fat gave a positive aldehyde reaction and a blue luminescence on exposure to the mercury-vapour lamp. Sheep's cheese thus differs from that of the cow chiefly in its albumin content and in the properties of the fat, which is distinguished by its low refractive index, high ratio of soluble to insoluble volatile fatty acids, and by the high saponification value. The microscopical flora of sheep's cheese consists chiefly of *Oidium lactis*, together with *Penicillium glaucum*, *B. lact. acid.* Leichm., and *B. casei.*

B. PLATON, E. HAGLUND and E. SÖDERBERG. **Influence of the dilution of whey on the consistency and acidity of cheese.** Sweden, *Centr. Exp. Sta. Medd.* **358**, 1929. (*Chem. Abst.* **24**, x, 2509, May 20, 1930.)

Experiments were made on the effect of dilution on the whey for cheese which were salted either directly or in brine. Curdling was brought about by the addition of 0·1 to 1 per cent. pure culture. Controls were also run so that four series in all were compared in which the dilution varied from 10 to 50 per cent. *p*H and the water content of the cheese were determined after 24 hours, 45 days and 120 days. For the last two periods of time the consistencies were determined, and for the 45-day period the salt content of the water. The cheese made with diluted milk were softer, more pliable and more elastic than those made from undiluted milk and the difference became more noticeable the greater the dilution. The *p*H values were greater for the diluted milks, and increased with the degree of dilution. The *p*H is low 24 hours after curdling, but it increases with age. The higher the value of the *p*H the softer did the cheese become. The optimum of added water is determined by the content of lactic acid bacteria and the manner of salting, and may vary from time to time, but since these factors primarily influence the *p*H, a determination of the

*p*H serves as a valuable means of control. A method of determining *p*H by means of a Hellige comparator was worked out. Bromcresol green gives values identical with those obtained by electrometric measurements. For fresh cheese the *p*H should be increased by a correction of 0·2 *p*H and for ripe cheese by 0·1 *p*H, when methyl red is used as the indicator.

BIOCHEMISTRY

I. Matsuo. **Milk. IV. The effect of heating on the constituents of milk.** *Osaka J. Med.* **28**, 555–62, 1929. (*Brit. Chem. Abst.* B, p. 528, June 13, 1930.)

Raw and sterilised milk contained respectively: total nitrogen 0·47, 0·449; albumin 0·410, trace; globulin 0·022, 0·014; caseinogen 0·370, 0·398 per cent. Human milk (4–5 days after parturition) contained 1·41–1·66 mg. of ferric oxide per litre, in 13–44 days 1·12–1·57 mg., in 201–218 days 1·05–1·43 mg.; raw and boiled cow's milk contained 2·02 mg. (0·33 mg. only in a glass receiver); goat's milk contained 1·17 mg.

A. F. Coca. **On the dialysability of proteins.** *J. Immunol.* **19**, iv, 405–10, October 1930.

The author claims to have shown that egg pseudoglobulin, ovoalbumin and ovomucoid are dialysable through collodion sacs, parchment diffusion shells and a fish air bladder. Less than 1 per cent. dialyses through the membrane in 5 days; the value of 70 per cent. reported by L. F. Loeb was probably due to errors in technique. J. G. Davis

M. M. Clayton. **The comparative value of different food proteins for reproduction and lactation in the rat. II. Milk, eggs, and meat.** *J. Nutrition*, **3**, 23–38, July 1930.

The rations were planned to contain 15–20 per cent. protein and 20 per cent. fat. Milk was fed only in dried form. A destructive effect on vitamin E was noticed on feeding with lard, or with large quantities of cod-liver oil. This was prevented by feeding the oil daily in small quantities. Other difficulties in the investigation included the ruling out of such factors as quality and assimilability of protein and amount and composition of mineral matter. Dried milk was found inferior to egg in vitamin E content but superior to beef, liver, and kidney. The amount of vitamin B present was of more importance for lactation and an alcoholic extract of wheat germ (ether extracted) improved lactation on the milk and meat diets. W. L. Davies

B. Sure. **Dietary requirements for fertility and lactation. XXII. Further Studies of the rôle of milk fat in fertility and lactation.** *J. Nutrition*, **2**, 485–9, May 1930.

The effect on fertility and lactation of the increase of small amounts of butter fat to a ration satisfactory for growth in the rat was studied by estimating the percentage of young weaned in the fifth generation. A minimum of 9 per cent. butterfat added to a ration of two parts whole wheat and one part skim milk powder produced optimum results in both fertility and lactation. One of the limiting factors in all the milk diets studied from the lactation standpoint was vitamin B.

W. L. Davies

G. C. Supplee, O. D. Dow, G. E. Flanigan and O. J. Kahlenberg. **A comparative study of liquid and dry milk as anaemia-producing diets.** *J. Nutrition*, **2**, 451–69, May 1930.

The production of nutritional anaemia in rats by feeding with raw milk and reconstituted dried milk of normal copper and iron content has been confirmed. Reconstituted dried milk containing increased iron content resulting from contact

with the drying plant prevented the development of anaemia and cured anaemia in rats. Complete recovery from anaemia occurred when the dried milk contained four times as much iron as normal milk, but the recovery was only partial when only twice the amount of iron was present. Supplementing the milk by inorganic addition of iron and copper did not furnish an appreciable degree of protection against anaemia. The results are not conclusive in showing that the copper content of milk is an important factor concerned in the development of anaemia in the rat. W. L. Davies

M. E. F. Crawford, J. Golding *et al.* **The fat soluble vitamins of milk.** *Biochem. J.* **24**, iii, 682–91, 1930.

The results obtained in these experiments suggest definitely that the entire vitamin A and vitamin D content of milk is associated with the milk-fat. There are no indications that these vitamins are more concentrated in one fraction of the milk fat than in another, nor that there is a significant loss in either vitamin due to separating or churning, and consequently by testing the butter it is possible to obtain the approximate relative potency of milk. These experiments further suggest the utility of the Soames and Leigh-Clare method for the determination of vitamin D.

Authors' Summary

H. Steenbock *et al.* **Fat soluble vitamins. XXVIII. The antirachitic value of cow's milk as modified by exposure of the cow to sunlight and to radiation from a quartz mercury vapour lamp.** *J. Biol. Chem.* **87**, 103–26, May 1930.

Daily exposure of cows to sunlight or artificially generated ultra-violet radiations has little if any effect on the antirachitic potency of milk. The results stand in marked contrast to earlier observations with goats. There was no improvement in milk yield or butterfat secretion. The superior quality of summer milk and butterfat therefore depends on factors other than insolation of the cow. W. L. Davies

H. Steenbock *et al.* **Fat soluble vitamins. XXIX. Is antirachitic activation induced by ultra-violet radiations a panacea for negative calcium balances?** *J. Biol. Chem.* **87**, 127–37, May 1930.

Direct exposure of a goat to radiations from a mercury vapour lamp increased the antirachitic value of its milk. This was in direct contrast to the effect on cow's milk. It was observed that the goat ultimately showed a decided negative calcium balance although a slight improvement in calcium retention was observed when the period of irradiation commenced. A pronounced negative calcium balance was accompanied by restlessness of the animal. It is therefore possible that the excretory elimination of calcium may be controlled neurologically as well as by the antirachitic factor. Antirachitic activation cannot be considered a panacea for a disturbed calcium balance. W. L. Davies

H. Steenbock, E. B. Hart, F. Hanning and G. B. Humphrey. **Fat soluble vitamins. XXX. The antirachitic value of cow's milk as modified by the feeding of irradiated yeast.** *J. Biol. Chem.* **88**, 197–214, August 1930.

50 g. of irradiated yeast fed to cows increased the antirachitic potency of milk. 10 g. of such yeast fed daily caused a slight increase in the antirachitic potency of the butterfat, an effect not clearly demonstrable in the milk. The effect of 10 g. was equivalent to that of 180 g. of cod-liver oil. The feeding of irradiated yeast may be considered as a practical measure for the production of milk of standard antirachitic potency. Feeding 200 g. of yeast did not lower milk yield nor fat content.

W. L. Davies

A. D. Pratt. **Does the nickel dissolved from the container during pasteurisation catalyse the destruction of the vitamins of milk?** *J. Nutrition*, **3**, 141–56, September 1930.

Milk pasteurised in a nickel container was found to have a nickel content of 15 parts per million. By comparing milk pasteurised in glass vessels with that pasteurised in nickel vessels it was found that vitamin A was not appreciably destroyed in either case. Partial destruction of vitamin B and C occurred during pasteurisation, but nickel did not increase the destruction. The results are statistically interpreted. W. L. Davies

C. Barthel. **Ripening process of cheese.** *Svensk. Kem. Tids.* **42**, 28–39, 1930. (*Chem. Abst.* **24**, xi, 2809, June 10, 1930.)

An address on the chemistry and mycology of cheese ripening, including tables of N partition.

H. Zeller, W. Wedemann, L. Lange and E. Gildemeister. **So-called low pasteurisation of milk.** *Arb. Reichsgesundh. Amt.* **61**, 1–72, 1929. (*Brit. Chem. Abst.* B, p. 528, June 13, 1930.)

Chemical and bacterial changes are recorded; the acidity is diminished, albumin and globulin are partly coagulated, the casein is altered, the catalase diminished, and the diastase destroyed.

A. C. Fay. **The normal limits of variation of the methylene blue reduction test.** *J. Agric. Res.* **40**, ix, 855–62, May 1, 1930.

From the results of a relatively large number (75–100) of comparative tests carried out on each of 19 samples of milk the author concludes that the normal variations are so small that the possible error in the reduction time for any one sample is not large enough to have any significant influence on the grading of that sample, even though the coefficient of variability becomes greater as the reduction time increases. The simplified methods of making the test (*i.e.* the use of apparatus that has merely been boiled, the use of different types of vessels for the measurement of the milk, the use of tablets for the preparation of the methylene blue solution, slight variations in the measurements of the amount of solution used) have increased its practicability, but have not seriously impaired its value for the grading of milk.
E. R. Hiscox

PHYSIOLOGY

C. Porcher and L. Maynard. **La graisse du sang et la graisse du lait pendant la lactation.** (Blood fat and milk fat during lactation.) *Le Lait*, **10**, xcvi, 601–13, June–July 1930.

In this paper the authors describe the determination of the fat per cent. in blood. They find that the fat per cent. in blood is constant in each case but varies with the individual and that it increases and remains constant during lactation, and is not due to the fat fed. The ratio fatty acid to unsaponifiable fat in blood is approximately a constant, varying from 1·25 and 1·40 to 1. They suggest that the fatty acid per cent. in blood is an index of the milk fat producing qualities of the cow.

Alongside their tabulated results they give the milk and fat yields and per cent. fat, both unsaponifiable and fatty acid. J. D. Garrard

W. O. Nelson and J. J. Pfiffner. **An experimental study of the factors concerned in mammary growth and in milk secretion.** *Proc. Soc. Exp. Biol. Med.* **28**, i, 1–2, October 1930.

The initiation of milk flow is caused by two separate factors (*a*) development of the mammary glands to a proper state, and (*b*) initiation of secretion. The former is brought about by an ovarian factor (*e.g.* lipid extract of corpora lutea) and the latter by the pituitary principle (anterior pituitary lobe extract). W. L. Davies

D. Rapport. **The interconversion of the major foodstuffs.** *Physiol. Rev.* **10**, iii, 349–473, July 1930.

L. S. Palmer, W. S. Cunningham and C. H. Eckles. **Normal variations in the inorganic phosphorus of the blood of dairy cattle.** *J. Dairy Sci.* **13**, iii, 174–95, May 1930.

The inorganic blood phosphate of 25 cows of all ages was determined by Brigg's modification of the Bell-Doisy method. The tests were made in periods of three consecutive days, and although the conditions were presumably identical, very considerable day to day variations in the P of the plasma were observed (up to ± 123 per cent., the maximum analytical error being ±10·37 per cent. and the usual error less than ± 3 per cent.). Some evidence of hour-to-hour variation was obtained so that the following factors were studied: *Feeding* caused a slight rise (5·7 per cent.) in 6 out of 10 cows, at end of ¾ hour. *Drinking* caused a very slight fall (3·6 per cent.) in every case, at end of ½ hour. *Exercise* caused a marked rise (6·34 per cent. just after) followed by a still more marked fall (22 per cent.) at end of ½ hour, and usually a further fall (4·9 per cent.) in next ½ hour.

The figures given above refer to the mean significant change in blood phosphate over and above the maximum analytical error, expressed as percentage of mean blood phosphate. *Parturition.* There was a fall on the day before parturition (may be as much as 3·2 mg. P per 100 c.c.) which usually persisted for several days. In all cases examined, the calf's blood contained more inorganic P than that of its dam. *Age.* The values were lower in first week, increasing up to 2 months with a gradual tendency to rise up to 6 months. Average from 0 to 185 days old was 7·26 mg. per 100 c.c. There was a slow decline after 6 months to the average value for mature cattle (5·91 mg. per 100 c.c. which was reached at about 18 months). G. L. Peskett

M. G. Bertrand and Y. Beauzemont. **Sur les variations de la teneur en zinc des animaux avec l'âge: influence du régime lacté.** (Variations of the zinc content of animals with age: influence of milk diet.) *Ann. Inst. Pasteur*, **45**, ii, 247–50, August 1930.

The zinc content of a mammal is high at birth and falls continuously during suckling; it continues approximately constant during adult life and finally in old age rises, ultimately exceeding that at birth.

Values of 37 mg. per 100 g. dry matter at birth and 11 mg. per 100 g. dry matter for adult rats are given.

Milk is very poor in zinc, hence young animals whose suckling is continued for a long period may suffer from a zinc deficiency. J. G. Davis

H. Mai and A. Keller. **Zur Unterscheidung von Frauenmilch und Kuhmilch.** (Method for distinguishing human from cow's milk.) *Klin. Wchnschr.* **9**, 535–6, 1930. (*Physiol. Abst.* **15**, iv, 234, July 1930.)

The method consists in determining the chloride-lactose quotient. In cow's milk the average chloride concentration is 114 mg. per cent. and lactose 5·13 gm. per cent. The authors' chloride-sugar quotient is 2·23 ± 0·05. For human milk the figures are: chloride 34·5 mg. per cent., lactose 7·48 g. per cent., quotient 0·44 ± 0·015. From

these figures it is easy to detect in a mixture of the two milks the relative amounts of each.

L. K. Achrap and N. A. Pokdopaew. **Material zur Frage über die Sekretion der Speicheldrüsen beim Trinken von Milch.** (The secretion of the salivary glands caused by drinking of milk.) *Pflügers Arch.* **224**, 539–44, 1930. (*Physiol. Abst.* **15**, v, 296, August 1930.)

Apart from the salivary secretion of the large salivary glands due to the chemical properties of the food given, there is a salivary secretion which is due to the actual administration of food and is independent of the physical and chemical properties of the food given. The salivary secretion on taking milk is due (*a*) to the act of taking the milk, and (*b*) to the necessity of digesting the milk.

G. Massia and J. Rousset. **La structure du mamelon chez la femme.** (Structure of the nipple in woman.) *Bull. hist. à la physiol. et à la path.* **7**, 118–28, 1930. (*Physiol. Abst.* **15**, iv, 205, July 1930.)

A description is given of the minute structure of all the parts of the nipple. The authors regard the appearance and distribution of a certain type of cell in "dyskeratosis" as of great importance. In old women these cells are found in the epidermis, and in the walls of the sebaceous canals and the galactophore canals; in young women they are very scanty. They are especially numerous in the sebaceous canals, and are found in fairly young women who have been pregnant and lactated; possibly the cells migrate at this time.

DAIRY HUSBANDRY

J. B. Lindsay and J. G. Archibald. **Mineral supplements for dairy cows.** *J. Dairy Sci.* **13**, ii, 102–16, March 1930.

This paper is a final report on 6½ years' investigation of this subject carried out at the Massachusetts Agricultural Experiment Station. During the earlier years tri-calcic phosphate in the form of steamed bone-meal as a source of lime and phosphorus showed little, if any, advantage in supplying these elements in that form. In the last 2 years a mixture of 80 per cent. di-calcic phosphate and 20 per cent. carbonate of lime was added to a ration supposedly deficient in lime, with the following results: With one exception all the cows in a herd of 13 to 16 maintained their general condition well. The mature cows receiving the mineral supplement made somewhat greater gains in live weight than the non-mineral group, but the added minerals had little, if any, effect on the growth of the young cows and heifers. Neither the milk yield nor the composition of the milk was significantly affected. Reproductive troubles were not serious in either group, but the cows receiving the mineral supplement were a little nearer normal, and produced slightly better calves.

The authors emphasise that none of the differences between the groups were sufficiently striking to warrant a general recommendation as to the use of this mineral mixture. For cows with a milk yield averaging 5000 to 8000 lb. annually the need of a mineral supplement is not indicated, but for heavy yielders (10,000 lb. and upwards annually) it is probably good insurance to supply additional lime and phosphorus.

They express the opinion that the problem of mineral deficiency in rations is largely a regional one, and that where the roughage carries a reasonably high percentage of calcium and liberal grain feeding is practised, mineral supplements will not be necessary for the average cow. J. Mackintosh

W. B. Ellet, C. W. Holdaway, J. F. Eheart and L. D. Lasting. **Feeding hevea rubber seed meal for milk production.** *Virginia Agric. Exp. Sta. Tech. Bull.* No. **41**, April 1930.

Hevea rubber seed meal is a new high protein concentrate in the United States. It is the by-product from the kernels of the seed of the Para rubber tree (*Hevea brasiliensis*) after the extract of oil. The by-product comes as a cake from the pulp presses, and when ground forms a dry, friable and rather fine meal, having a pleasant odour. Experiments with this meal have been conducted with dairy cows to determine its value for milk production, and the digestibility of its constituents.

The results indicate that Hevea rubber seed meal may be used as a medium protein concentrate in rations for cows. It is palatable, neither laxative nor constipating in its physiological effect on the digestive system and is apparently equal to linseed meal (equivalent to English linseed cake or cake meal) for milk production. Up to 5 lb. per head daily may be given. The analysis and digestibility coefficients (in brackets) were as follows: moisture 9 per cent., crude protein 27·5 per cent. (71), ether extract 9·5 per cent. (92), crude fibre 15·0 per cent. (20), nitrogen free extract 33·5 per cent. (58), ash 5·4 per cent. J. Mackintosh

O. E. Reed and C. F. Huffman. **The results of a five year mineral feeding investigation with dairy cattle.** *Michigan State College Agric. Exp. Sta. Tech. Bull.* No. **105**, February 1930.

In this experiment the effects of a basal ration low in minerals (especially calcium), of mineral supplements in the form of bone flour, limestone rock (finely ground), raw rock phosphate (finely ground), and a complex mineral mixture and of alfalfa hay in place of timothy hay were studied. Seven lots of five heifers (all Holstein Friesians of similar age and breeding) were used and maintained under this experiment from the age of six months to five years. Data were obtained on the rate of growth, reproduction, health of cows and calves, milk and fat yields and the effect of the various supplements on appetite and on the bones and teeth. Numerous illustrations of the cows at various ages and of their calves are given, and interesting information is supplied on the rate of growth, breeding records, etc.

The results indicate that the general need for mineral supplements for dairy cattle under normal conditions may be greatly exaggerated. Mineral supplements supplying calcium are not greatly needed even when such feeds as timothy hay and other low calcium roughages are used. Raw rock phosphate to the extent of 1·5 per cent. of the grain mixture injured the health and teeth of the animals, perhaps by reason of its fluorine content; also the complex mineral mixture used caused harmful effects and injury to the teeth in the course of time. J. Mackintosh

R. A. Roberts. **The utilisation of grassland for milk production.** *J. Brit. Dairy Farmers' Assoc.* **42**, 29–40, 1930.

This article gives a description of the dairy pastures of England and indicates to what extent pasture can supply the dairy cow's requirements. It is pointed out that the temperate regions of the earth tend to provide foods—grass, cereal grains and root crops—higher in carbohydrates and poorer in proteins than are considered balanced for the needs of the dairy cow. The author shows how this protein shortage may be to a large extent overcome by management of grazing land, the early cutting of hay and the selection of suitable species of forage plants. The mineral content of pastures is also discussed. J. Mackintosh

J. A. Crichton. **Economic calf rearing.** Series **V**, 2, Government of Palestine Agric. Leaflets.

K. W. D. Campbell. **Milking at three eight-hour intervals as a means of investigating variations in the fat and solids-not-fat.** *J. Agric. Sci.* **20**, ii, 213–32, April 1930.

The object of this investigation was to collect evidence on the effect of thrice-daily milking on the yield of individual cows, and on the effect of milking at three eight-hour intervals on the percentage and weight of butterfat. Twelve cows in the herd at the University of Reading farm were milked thrice daily, at the hours of 6 a.m., 2 p.m. and 10 p.m. throughout a complete lactation; at each milking the milk yielded by each cow was weighed, sampled and the percentages of fat and solids-not-fat determined. The cows were all home bred Dairy Shorthorns, which calved between November 29th, 1927 and May 3rd, 1928, and of different ages; namely, 1st calf, 3, 2nd calf, 3, 3rd calf, 2, 4th calf, 2, and 5th calf, 2. The management was on uniform lines throughout the period.

It was found that the nine cows increased in milk yield when milked thrice daily by an average of 19·3 per cent., the individual yields showing a range from a decrease of 21·1 per cent. to an increase of 41·8 per cent. The calculated increase obtained from the three first-calf heifers was 5·6 per cent. These comparisons are made on the milk yields after standardisation by the methods suggested by Sanders. The effects of intervals of varying length between the milkings on the yield and percentage of fat is discussed, and tables are given showing the variation in milk yield and fat percentage from day to day. The author suggests that some factor operates at night tending to lower the rate of fat secretion and that this factor operates whether the night interval be long or short.

(No reference is made in the test to the solids-not-fat content of the milk, nor to the possible influence of the milkers on the yield and fat content of the milk.)

J. Mackintosh

H. T. Cranfield. **Effect of abnormal weather conditions on the quality of milk.** *J. Min. Agric.* **37**, iv. 347–50, July 1930.

During the 12 months ending September 1929 only 16·8 inches of rain fell in South Notts. This resulted in the diminution of growth of grass with consequent depression of milk yields. At the same time bulk milk was frequently low in non-fatty solids, the total number of samples containing less than 8·5 per cent., being 75 or 16 per cent. of the total in July and 49 or 45 per cent. of the total in September. July is most frequently the month in which non-fatty solids fall below 8·5 per cent.

It has been observed by other workers that the effect of drought is to depress yields, raise fat percentages and depress non-fatty solids.

(In 1928 more than 10,000 samples of milk from individual cows were tested by lactometer at Reading for specific gravity and non-fatty solids. The maximum number of samples below 8·5 per cent. occurred in July 1928, being 33 per cent. of the total for that month. The rainfall for 1928 was one inch lower than the average for the preceding ten years.)

K. W. D. Campbell

R. C. Gaut and C. L. Walton. **The ox warble flies and their control.** *J. Brit. Dairy Farmers' Assoc.* **42**, 16–28, 1930.

The authors give a concise description of the life-history of the ox warble flies, and of the methods of treatment formerly recommended, then describe more fully the experiments carried out by the Worcestershire County Council in 1928 and 1929. Various kinds of dressings were applied to the backs of the affected animals and the effects on the animal itself and on the larvae noted. The best results were obtained with a preparation known as Derris-soap wash, consisting of 1 lb. derris powder, ¼ lb. soft soap and 10 lb. water. Applications were made four times during the "warble season," *i.e.* March to June inclusive. The systematic and thorough use of

this wash will result in killing practically all the warbles and very greatly lessen number of flies. In areas where there is no importation of affected cattle the flies can be exterminated in a few years. J. MACKINTOSH

DAIRY INDUSTRY

A. BURR. **The water content of cheese.** *Landw. Jahrb.* **68**, 172–3, 1928. (*Chem. Abst.* **24**, xii, 3063, June 20, 1930.)

The H_2O content of fresh and cured cheese is given. Seven varieties have been studied.

S. ORLA-JENSEN and E. W. SIVERSTEN. **Om Vandsterilisering.** (Sterilisation of water.) *Maelkeritidende*, **43**, xxxviii, 799–801, September 19, 1930.

Cheap and effective methods of sterilisation of water are of interest to the dairy industry, since bad water is a source of trouble. The author has found that the addition of HCl or NaOH, and subsequent neutralisation is not effective for naturally infected water. If only small quantities are needed in the dairy, heating the water is the easiest method. Chlorination gives good results if it has no influence on the flavour of the butter, etc. E. R. HISCOX

O. LAXA. **Undersøgelser af Jonas Nielsens Sterilisator.** (The Jonas Nielsen steriliser.) *Maelkeritidende*, **43**, xxxvi, 765–9, September 5, 1930.

An account, with diagrams, is given of the recent modifications of the Jonas Nielsen steriliser. The milk is allowed to flow through tubes in cylinders I–IV where it is gradually warmed to a temperature of 70°–80° C. by means of the outflowing milk. In cylinder V it is heated to a temperature of 108°–110° C. or 128°–130° C. by means of steam after which it returns through cylinders IV–I losing heat to the inflowing milk, and is finally cooled to a temperature of 80° C. by means of water and cooling mixtures in cylinders VI–VIII.

Bacteriological investigations of the milk at different stages show that when the milk is heated to 128°–130° C. in cylinder V it is very rapidly sterilised. When it is heated to 108°–110° C. the sterilisation is not complete in this cylinder, but may be assumed to be complete by the time the milk has returned to cylinder III. The pipes in the cooling cylinders VI–VIII must be very carefully cleaned and sterilised, otherwise heavy contamination with *B. coli* and other organisms may occur at this stage. The advantages of this method of sterilisation are discussed. E. R. HISCOX

C. E. SLAUGHTER. **Disposal of creamery wastes.** *Michigan Eng. Exp. Sta. Bull.* **18**, July 1928.

The author reviews the special features of creamery wastes and the legal position as to their disposal in Michigan. In order to determine the lines on which large-scale experiments should be based, he conducted a series of controlled laboratory tests with a 2 per cent. milk solution embodying the principles of tank storage, septic tanks, contact beds, controlled acidity tanks and chemical precipitation, with further treatment of the effluent in secondary tanks or sand filters.

The most successful processes appear to be either the use of lime in septic tanks or treatment with ferrous sulphate and lime with subsequent precipitation. Definite recommendations, based on laboratory evidence, are given for the disposal of wastes from isolated creameries, together with the estimated cost of operation. J. HOLMES

E. F. ELDRIDGE. **Studies on the treatment of milk products waste.** *Michigan Eng. Exp. Sta. Bull.* **24**, July 1929.

This is a progress report of investigations conducted with an experimental plant at the Michigan State College. The methods of disposal were (1) chemical precipitation, (2) biological filtration of effluent from chemical precipitation, (3) biological treatment of raw waste on sand filter, (4) biological treatment of raw waste on gravel filter. Detailed procedure and analytical results are given in each case. It is suggested that whilst no single system can be universally applied, properly controlled chemical precipitation with ferrous sulphate and lime gives a clarified effluent suitable for secondary treatment on a biological filter.

Results obtained with sand and gravel filtration of the raw waste were encouraging, but further investigations appeared advisable. J. HOLMES

E. F. ELDRIDGE. **The treatment of milk products waste.** *Michigan Eng. Exp. Sta. Bull.* **28**, March 1930.

This paper describes a further study of creamery waste disposal by means of biological filtration through sand, gravel, brush and cinder mediums, together with a general review of the subject, and the main features to be considered before deciding on the most efficient method in the case of any particular creamery. Further investigations are in progress dealing mainly with the economical aspects of the subject. J. HOLMES

REVIEW

A. G. HOUSE. **The Bacteriological Control of Milk. A practical guide for media preparation and milk testing.** Cambridge University Press, 1931. (3*s.* 10*d.* post free from the National Institute for Research in Dairying.)

In a foreword Dr R. Stenhouse Williams refers to Mr House's long experience in media-room management and routine bacteriological analysis. The fruits of this long experience are offered in a concise manner, each chapter containing an excellent summary of the methods described. Many labour- and time-saving hints are given, these in themselves making the book a valuable addition to the laboratory shelf.

There are three main sections: (1) The preparation of glassware, (2) The preparation of media, and (3) Methods of milk testing—the official plate-count method being dealt with in full detail.

An excellent appendix contains a complete list of laboratory furnishings and apparatus required to carry out from 10 to 100 routine tests weekly. Attached to the appendix are two very practical ground plans for milk-testing laboratories, both of which can be adapted to existing buildings.

The monograph is eminently suitable as a book of reference for workers who have previously undergone a course of training in bacteriological technique, and is invaluable to anyone who contemplates the installation of a control laboratory.

(In naming this monograph the main title might well have been omitted, as no attempt is made to suggest methods by which any actual control can be practised over milk supplies.) L. J. MEANWELL

STATISTICS

Number of Dairy and Other Cattle

The following table shows comparative figures of the dairy herd, and of other cattle, in the principal countries within the British Empire in which dairying is of major importance. The figures are in each case the latest available, and are generally of a preliminary nature, but it should be noted that the estimates of livestock relate to different dates in the various countries. It should also be noted that classification of cattle into the dairy herd and other cattle is not a matter of uniform practice throughout the Empire, but the figures give a fairly accurate indication of the extent of the dairy herd and of any change in comparison with the preceding census.

Countries	Year*	Cows and heifers in milk or in calf	Other cattle	Total cattle
		000 head	000 head	000 head
England and Wales...	1930	2670	3176	5,846
	1929	2713	3245	5,958
Scotland	1930	452	781	1,233
	1929	454	779	1,233
Northern Ireland ...	1930	256	417	673
	1929	264	436	700
Irish Free State ...	1930	1311	2727	4,038
	1929	1307	2830	4,137
Canada	1930	3683	5254	8,937
	1929	3685	5140	8,825
Australia	1928–29	2467	8834	11,301
	1927–28	2422	9195	11,617
New Zealand ...	1930	1424	2297	3,721
	1929	1371	2075	3,446
Union of South Africa	1929	Not available		10,518†
	1928	Not available		10,478
Kenya	1930	68	159	227‡
	1929	61	157	218

* Estimates relate to June 1 except New Zealand (January 1), South Africa (August 31), Kenya (July 31); for Australian States estimates refer to December 31 except New South Wales (June 30 following).

† Including 6,773,000 cattle on farms owned by Europeans.

‡ Relates solely to European-owned cattle; the estimated total number of native-owned cattle in Kenya in 1930 was 4,966,000.

TRADE IN DAIRY PRODUCE

The following tables give details of the trade in butter and cheese of the principal countries handling these products during 1930, with comparative figures for 1929. The particulars are based on information published by the International Institute of Agriculture, Rome, brought up to date, where necessary, from official sources.

Imports and exports of butter

Countries	Exports		Imports	
	1930	1929	1930	1929
Exporting countries	000 lb.	000 lb.	000 lb.	000 lb.
Austria	4,112	2,211	545	1,098
Denmark ...	372,558	350,620	1,389	1,424
Esthonia ...	31,010	27,247	—	—
Irish Free State...	58,815	62,836	3,391	4,621
Finland	37,726	36,610	7	2
France	12,095	16,722	12,924	9,753
Hungary... ...	3,430	1,191	40	143
Latvia	40,630	32,624	49	49
Lithuania ...	16,219	9,004	—	—
Netherlands ...	92,394	104,325	4,396	4,469
Poland	26,714	33,248	29	112
Sweden	58,857	54,977	18	24
U.S.S.R.... ...	17,229*	56,009	—	—
Argentina ...	48,866	37,547	—	—
India	551	522	282	229
Syria and Lebanon	1,916†	2,116	170†	238
Australia ...	126,411	102,919	2	4
New Zealand ...	209,771	183,639	—	—
Importing countries				
Belgium	2,648	2,877	22,412	9,559
Czecho-Slovakia	694	717	714	836
Germany ...	578	337	293,560	296,230
Greece	—	—	1,420	1,537
Italy	1,843	1,651	3,115	1,909
Norway	236	1,191	1,530	1,351
Switzerland ...	42	159	18,786	16,649
United Kingdom	21,028	14,839	764,782	717,539
Canada	1,179	1,400	38,605	35,929
U.S. America ...	2,967	3,913	2,471	2,773
Ceylon	—	—	723	736
Java and Madura	—	—	7,557	7,712
Japan	—	—	611	503
Algeria	82	64	3,232	2,207
Egypt	42	29	2,417	1,649
Tunis	13	18	829	717

* Data to September 30. † Data to November 30.

Imports and exports of cheese

Countries	Exports		Imports	
	1930	1929	1930	1929
Exporting countries	000 lb.	000 lb.	000 lb.	000 lb.
Czecho-Slovakia	8,274	7,050	2,963	3,349
Denmark ...	12,626	14,513	809	648
Finland	4,683	4,837	35	44
Italy	80,974	71,803	12,562	13,975
Lithuania ...	1,960	1,299	11	11
Netherlands ...	206,739	211,237	1,510	1,446
Norway	1,380	1,347	750	840
Poland	3,267	3,907	1,074	1,351
Switzerland ...	65,925	69,737	4,251	3,437
Yugo-Slavia ...	4,583	4,890	300	370
Canada	66,955	92,945	1,779	2,103
Australia ...	7,273	5,135	150	597
New Zealand ...	201,863	197,552	4	7
Importing countries				
Austria	4,482	2,923	5,637	5,717
Belgium	875	899	51,106	46,456
France	38,921	40,609	65,524	52,173
Germany ...	5,410	4,919	137,459	146,570
Greece	262	355	2,302	3,314
Hungary... ...	93	40	386	547
Irish Free State	194	154	2,350	2,410
Sweden	—	—	1,470	1,420
United Kingdom	8,927	9,976	348,592	335,331
U.S. America ...	2,130	3,020	68,313	76,382
India	7	7	1,133	1,237
Java and Madura	—	—	1,671	1,821
Syria and Lebanon	128*	196	582*	703
Algeria	212	194	10,397	8,468
Egypt	60	123	7,553	6,563
Tunis	29	13	1,746	1,695

* Data to November 30.